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ORGANISMS, By A. B. MACALLUM

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ON THE CYTOLOGY OF NON-NUCLEATED
ORGANISMS

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ON THE CYTOLOGY OF NON-NUCLEATED ORGANISMS

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In the following pages I have endeavoured to describe the results of observations which I have made during the last five years on the structure of certain types of non-nucleated organisms. These studies are not as complete as I would wish them to be, especially in the case of Bacteria, for only a few of the forms of the latter accessible to me were of sufficient size to enable me to employ all the micro-chemical methods used in the case of the Cyanophyceæ and *Saccharomyces*, but the results described may prove of service to other investigators in the same line, and at the same time stimulate the employment of comprehensive methods of technique in the study of non-nucleated organisms. It is doubtful if the ordinary or more complicated methods of staining which have been used in this department give results which are of the morphological value possessed by results obtained in this way in more highly specialized animal and vegetable cells. The existence of a nucleus in these low forms of life, if not denied by the great majority of observers is at least in dispute. If it is absent what takes its place? Is chromatin present or does there exist in them an analogous substance? As there are in many animal and vegetable cells other substances which may take up dyes, more especially of the aniline kind, it is obvious that staining methods alone are not sufficient to enable the observer to determine the solution of questions like these in regard to the lowest forms of life. A more satisfactory determination of such questions can be made only with methods which comprehend micro-chemical reactions of definitely ascertained values.

It may be pointed out also that it is in these low forms of life that we must look for a key to the secret of the origin of the cell nucleus, as well as for data to determine the morphological character of the primal life organism. It is of course suspected by many that in *Saccharomyces* the

cell as it is now found does not represent what it once was, that it is the product of the degradation of a higher form of cell. In that case it may be contended that one cannot obtain from the present structure of the cell data which can be of service in ascertaining what the structure of the primal cell was. If the yeast cell, which, as I claim, is non-nucleated, is the product of the degeneration of a higher type of cell, of, for example, a nucleated cell, an examination of it may still serve a purpose in assisting in determining the origin of the cell nucleus, for we can then ascertain how in the yeast cell its surplus of chromatin is distributed in the cytoplasm in all conditions, and this may possibly indicate to us in what manner the original non-nucleated cell disposed of the excess of the analogous chromatin compound.

From this point of view, therefore, the employment, not only of methods of staining, but also and particularly of micro-chemical processes in the investigation of these low forms of life is absolutely necessary if results of any value are to be obtained. These methods I have endeavoured to employ to a certain extent and this constitutes the excuse for adding one more contribution on the subject.

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THE CYANOPHYCEÆ.

I.—LITERATURE.

The first to examine carefully the structure of the Cyanophyceæ was Schmitz,¹ who found in the cells of *Glaucocapsa polydermatica* a homogeneous central portion which stained with hæmatoxylin and represented, as he thought, the nucleus of the cell. He found also that there were in the cell substance a number of spherules of unknown composition, to which he applied the term "Schleimkügelchen." In the cells of *Oscillaria princeps*, after being stained with hæmatoxylin, he observed a dark, spherical, somewhat excentrically placed body which he regarded as a nucleus, but he was unable to demonstrate its presence in all preparations of this form.

Further observations,² however, led Schmitz to regard the cells of Cyanophyceæ as non-nucleated and the supposed nuclei of *Glaucocapsa* as simply very large chromatin granules which react with hæmatoxylin like the chromatin granules of the nuclei of higher organisms. He distinguished in *Oscillaria princeps* a peripheral, finely punctated zone in each cell which stained with hæmatoxylin less deeply than the central portion.

In a later work he denies the existence of a chromatophore and claims that the functions performed in the special parts of highly differentiated cells, are in the Cyanophyceæ the property of the cell protoplasm generally.

Tangl³ also was unable to determine the presence of a nucleus, but he found a chromatophore in *Plaxonema Oscillans*. According to Wille,⁴ however, a nucleus exists in *Tolypothrix lanata*. After being stained with a dilute or a concentrated hæmatoxylin solution, preferably the latter, the nucleus appeared faint blue, the nucleolus, on the other hand, intense blue, while the remaining cell contents were scarcely stained. In the dividing cell he observed two nuclei, each with a

¹ "Untersuchungen über den Zellkerne der Thalophyten," Sitzungsber. der Niederrh. Gesell. für Natur- und Heilkunde zu Bonn, Sitz. vom August 4, 1879, p. 355.

² "Untersuchungen über den Struktur der Protoplasma und der Zellkerne der Pflanzenzellen," Ibid, Sitz. am Juli 12, 1880, p. 159.

³ "Die Chromatophoren der Algen," Bonn, 1882, quoted by Deinema.

⁴ "Zur Morphologie der Cyanophyceen," Wien, 1883.

⁵ "Ueber die Zellkerne und die Poren der Wände bei den Phycochromaceen," Berichte d. deutsch. bot. Gesell., Vol. I, 1883, p. 243.

nucleolus, and in another stage of division he found one nucleus with two nucleoli. The next observer, Lagerheim, found no nucleus in *Glaucozystis Nostochinearum*. The chromatophore in the young cells of this form is, according to his description, in the form of a band or thread surrounded by the colourless elements of the cell, but in the older cells it is composed of a large number of small granules which form a membrane lying at some distance from the cell wall and enclosing the colourless cell substance.

Reinhard² found in *Oscillaria major* (?) when fixed with picric acid and stained with hæmatoxylin, a large granular nucleus with large granules as nucleoli. The protoplasm contains large and small granules, the former constituting the chromatophores. Hansgirg, although maintaining the existence of a chromatophore and nucleus in *Chroodactylon Wolleanum*, a unicellular blue-green form, held that in the thread-like Cyanophyceæ there is neither a nucleus nor a chromatophore and that the cell protoplasm discharges the functions of both.

Borzi⁴ found neither a nucleus nor a chromatophore. He distinguished by micro-chemical methods, amongst the granules present, a kind the examples of which are partly imbedded in the protoplasm and partly applied to the cell wall. These granules are formed of a gelatin substance, which he believes replaces starch in these forms, and which he names cyanophycin. The granules, he believes also, are secreted in dividing cells by the young transverse septa. In *Nostoc*, *Anabæna*, *Spermosira*, *Cylindrospermum* and *Sphaerozyga*, the protoplasm of two adjacent cells is connected by fine strands, sometimes of plasma, at other times of cyanophycin, which pass through openings in the transverse septa. These openings are specially marked in heterocysts in which they closed by plugs of cellulose, protein or cyanophycin. Similar perforations were observed in the transverse septa in *Oscillariæ* and Borzi explains their function as that of uniting the protoplasm of all the cells to enable them to act as a unit in the case of movement.

In his first publication⁵ on the subject of a nucleus in the Cyanophyceæ, Zacharias stated that he found this organ in the terminal cells

¹ "Ein neues Beispiel des Vorkommens von Chromatophoren bei den Phycochromaceen," *Berichte d. deutsch. bot. Gesell.*, Vol. II, 1884.

² "Algologische Untersuchungen, I. Materialien zur Morphologie und Systematik der Algen des Schwarzen Meeres," Odessa, 1885, (Russian). Abstract given by Deinema.

³ Ein Beitrag zur Kenntniss von der Verbreitung der Chromatophoren und Zellkerne bei den Schizophyceen (Phycochromaceen)," *Berichte d. deutsch. bot. Gesell.*, Vol. III, 1885.

⁴ Malpighia, Anno I, 1886. Abstract in *Bot. Centralbl.*, Vol. XXXII, p. 35, 1887.

⁵ "Beiträge zur Kenntniss der Zellkerne und der Sexualzellen," *Bot. Zeitung*, 1887, Nos. 18-24.

of *Tolypothrix* after treatment with a digestive fluid, and extraction with alcohol and ether, and on examination in a solution of hydrochloric acid of 0.3 per cent. strength. In this case the nucleus was demonstrated by the nuclein lustre produced. The nuclein was extracted with a soda solution of 0.05 per cent. strength from threads which had previously been submitted to digestion. He found also in a species of *Oscillaria*, after treatment with digestive fluids, a large nucleus, containing nuclein network, in every cell. From such preparations the nuclein was removed by extraction with very dilute soda solutions.

Scott¹ has described the occurrence of nuclei and nuclear division in *Oscillaria* and *Tolypothrix*. The nuclei were rounded, sometimes contracted, and presenting evidences of a fibrillar structure like that observed in the "skein" stage of the nuclei of more highly specialized cells. Scott regarded this structure as due to the division of the nucleus, as it was accompanied by division of the cell as a whole, and he found in a few preparations indications of colourless striæ connecting the portions of the nuclear structure, and suggesting the idea of "achromatin fibres." In some cells the nuclear fibrillæ were broken up into smaller portions which resembled the chromatin segments of division in ordinary cells.

A second publication² by Zacharias contained the results of a very comprehensive research on the structure and micro-chemistry of the cells of the Cyanophyceæ. He found in all the forms examined by him that only the peripheral portion of the cell substance is coloured, the central portion appearing colourless. The coloured substance was more abundantly present on the lateral than on the transverse walls. He was unable to determine the existence of a chromatophore. Vacuoles were not present in the normal cells, but made their appearance in threads which were observed dying under the microscope. In *Oscillaria*, after several days' culture, with the exclusion of light, vacuoles of an undetermined character were found to occur. In the colourless central portion of the cells articulated or granulated structures, with one or two bodies which possessed the appearance of nucleoli, could be recognized in favourable cases. The nucleoli-like elements were colourless, spherical, not sharply circumscribed, and appeared as if their peripheral substance was of a denser consistency than their centre. A portion of the central body of the cell was found to be soluble in artificial gastric juice, the undissolved residue being composed of two substances, or of one only.

1. "On Nuclei in *Oscillaria* and *Tolypothrix*," The Journal of the Linnean Society, Botany, 1887, Vol. XXIV, p. 188.

2. "Ueber die Zellen der Cyanophyceen," Bot. Zeitung, 1890, Nos. 1-5.

One of these two, related to plastin in its characters, was always found to be present; the other, denominated the "central substance" (Central Substanz), which might be absent, was found to be similar to the nuclein of higher organisms in many respects. The substance of the nucleoli-like elements was not found to differ from that of the nucleoli of higher plants.

Zacharias discussed the question whether the central colourless body represents a nucleus, contrasting it with the nuclei of higher forms of vegetable life, and pointing out that in the Cyanophyceæ nuclein may be present in some cells of a thread, but wholly absent in others, a condition not observed in the tissues of more highly organized vegetable forms. Nuclein was wholly absent in the dividing cells of Cyanophyceæ, while division in the cells of higher organisms is always preceded by an increase in the quantity of chromatin or nuclein. On account of such contrasts Zacharias regarded it as uncertain whether the "central substance" of the Cyanophyceæ represents the nuclein of other organisms, and he concluded that the central body is different in all its relations from a nucleus. The absence of a nucleus is, he believes, associated in some way with the absence of a sexual process.

Bütschli,¹ in his studies on the structure of Bacteria and Cyanophyceæ, came to the conclusion that in both the same type of structure prevails. He found in the cells of the Cyanophyceæ a peripheral coloured zone of cytoplasm which stains feebly with hæmatoxylin, enclosing a colourless "central body" which takes the hæmatoxylin stain more strongly. The two structures are vesiculated, that is, provided with a honeycomb-like structure (Wabenstruktur). In the cytoplasm hæmatoxylin reveals, by the red colour which it gives them, a number of granules, termed by Bütschli the "red" granules, which are situated in the nodal points of the vesicles, and are more abundant and larger at the periphery of the central body than elsewhere. These are not to be found after the cells have been treated with hydrochloric acid and pepsin, but he attributes their disappearance to the digesting reagent, having deprived them of the power of taking up dyes, since they readily lose their power of staining when they are fixed with osmic acid, corrosive sublimate, or with the picro-sulphuric or chrom-osmic-acetic mixtures. He is inclined to regard them as composed of a substance like chromatin in many respects. Other granules of a different nature were found in the peripheral cytoplasm, and more particularly near the transverse walls in *Oscillaria*, which exhibited an affinity for eosin, but

¹ "Ueber den Bau der Bacterien und verwandter Organismen," Leipzig, 1870.

were wholly unaffected by hæmatoxylin. The colouring matter of the cell is disposed in the framework of the vesicles of the peripheral zone and not in the fluid filling the vesicles (enchylema).

Regarding the nature of the central body Bütschli speaks positively. It is, he maintains, the homologue of the nucleus of higher forms. Zacharias had at first held this view, but withdrew it,¹ because he found that in the central body, in many Cyanophyceæ there is no nuclein, and that, further, when such cells divide, there may be no nuclein demonstrable in them, facts which are quite the reverse of those observed in the typical nuclei of other forms. Zacharias admits that in some animal and vegetable nuclei nuclein may be absent, and Bütschli points out the force of the admission, while he calls attention to the fact that the absence of mitotic phenomena is no indication that the central body is not a nucleus, for the macro-nuclei of Infusoria do not undergo mitosis, but direct division. When the cells of *Oscillariæ* were subjected to the action of artificial gastric juice the peripheral layer wholly disappeared in some cases, while, in others, portions only of it remained, but the "central body" was preserved, and resembled more markedly a nucleus, retaining also its capacity for absorbing hæmatoxylin.

Fischer,² in the year following the publication of Bütschli's paper, attacked the correctness of the methods of that observer, claiming that the occurrence of a peripheral layer in the cells of Cyanophyceæ and Bacteria is an artificial production, in fact due to plasmolysis caused by the fixing reagents and means used in the preparation of the organisms. Fischer's observations were made on a limited number of small bacteria and also upon unspecified *Oscillariæ*. In these the reagents employed caused the shrinkage of the cell protoplasm from the cell membrane and as the shrinkage was unequal, strands of cytoplasm were left extending between the shrunken mass and the cell wall to simulate a peripheral layer of vesiculated structure. This condition accounted for Bütschli's results. Fischer denied even the existence of a colourless central body in the Cyanophyceæ.

Bütschli's³ answer to Fischer's objections was that, admitting some reagents do produce plasmolysis in vegetable cells, this is by no means frequent, and treatment with the reagents which he used, viz., picro-sulphuric acid with or without osmic acid, the chrom-osmic-acetic mixture,

¹ Op. Cit.

² "Die Plasmolyse der Bakterien," Berichte der k. sächsl., Ges. der Wissensch., Mat.-Phys. Kl., 1891, p. 52.

³ "Untersuchungen über mikroskopische Schäume und das Protoplasma," Leipzig, 1892, p. 75-79.

and osmic acid, when properly employed, do not result in this condition. He pointed out further that the majority of the more recent investigators of the structure of the Cyanophyceæ had observed a colourless central body whose existence Fischer denies, and that the latter did not make sufficiently extended observations to justify such objections.

Deinega's¹ observations were made on *Oscillaria princeps*, *O. Froehlichii*, *Aphanizomenon flos-aquæ*, *Nostoc* sp., and *Scytonema* sp., and they dealt chiefly with the question of the presence of a nucleus and of a chromatophore, and with the nature of the granules in the cells of these organisms. According to Deinega, a chromatophore is present, and consists of a more or less perforated plate, apparently applied to the inner surface of the cell wall, the trabeculæ of the plate carrying the colouring matter, and running parallel, in the greater number of cases, with the trichome.

He found only one kind of granules, and these manifested a certain special affinity for picrocarmine. In *Oscillaria* they occur chiefly in the immediate neighbourhood of the transverse walls. They readily dissolve in dilute hydrochloric acid and in chloral hydrate, while they remain uncoloured in solutions of iodine. Deinega does not accept the view of Cohn² and Hansgirg³ that they are formed of paramylum, but he believes that they are composed of an isomer of starch.

In regard to the question of a nucleus Deinega is undecided. He repeated some of Zacharias' experiments with gastric juice, and found that in each cell of *Oscillaria*, after treatment with this reagent, the central body was provided with a nuclein lustre, but he attributes this to the remains of the chromatophore. He found, when examples of *Spirgyra* and other Algæ were similarly treated, their nuclei vanished, while the chromatophore remained and gave a nuclein lustre. The central body of the cell in Cyanophyceæ stained more deeply than the remainder of the cell protoplasm, and consisted chiefly of a collection of granules.

Zukal⁴ regards the central uncoloured portion of the cell as the cytoplasm, and the coloured peripheral part as the chromatophore, which, under very highly magnifying powers, appears finely punctated, the

¹ "Der gegenwärtige Zustand unserer Kenntnisse über den Zelleninhalt der Phycochromaceen," Bulletin de la Soc. impér. des Naturalistes de Moscou, Année 1891, p. 431.

² "Beiträge zur Physiologie der Phycochromaceen."

³ "Physiologische und Algologische Studien" (1877).

⁴ "Ueber den Zelleninhalt der Schizophyten," Sitzungsber. d. k. Akad. d. Wiss. zu Wien. Math.-Nat. Klasse, Vol. CI, p. 301, 1892.

latter fact suggesting that the chromatophore is formed of an exceedingly delicate reticulum. The granules, according to Zukal, are nuclei, and as such divide, the divisions not being followed necessarily by division of the containing cell.

Hieronimus¹ found a thin hyaline layer enveloping the cellular protoplasm, and applied to the internal surface of the cell membrane. The chromatophore is, according to his view, of fibrillar structure, and on it are arranged fine granules which appear to contain the chlorophyll. The phycocyan is dissolved in enchylema. The central body is formed of a wound fibril, and contains all the remaining granular elements, which he regards as crystals of the regular system. The substance of these crystals he calls cyanophycin, and though he recognizes that it does not correspond in its character to nuclein, yet he looks upon it as related to the chromatin and pyrenin of the higher organisms. The central body is, further, an open nucleus, that is, a nucleus without a membrane.

In reply to Zacharias² who criticized his observations, Hieronimus³ maintains that there is only one kind of granules in the cells of Cyanophyceæ, and that two kinds appear to be present because of the methods of preparation. He found that when the fixed cells were treated with ammonia vapour all the granules received a dark blue colour from hæmatoxylin, and that all the granules dissolved in diluted acid solutions, although they varied in the readiness with which they dissolved.

According to Palla⁴ the cell in the Cyanophyceæ consists of a colourless "central body," a coloured peripheral layer, the chromatophore, an external investing colourless layer, and possibly also a colourless zone (Plasmaschicht) situated between the chromatophore and the "central body." The "central body" reacts with dyes like a nucleus, but it contains no granules, and is homogeneous. Its division takes place through constriction. The granules in the cell are of two kinds: one composed of a substance, cyanophycin, soluble in dilute hydrochloric acid, and which stains pure blue with hæmatoxylin, found usually in the extreme periphery of the chromatophore, and representing the first assimilation product of the latter; the other, composed of a viscous substance, insoluble in dilute acids, and staining reddish-violet with hæmatoxylin.

¹ "Beiträge zur Morphologie und Biologie der Algen," Cohn's Beiträge zur Biologie der Pflanzen, Vol. V, p. 461, 1891.

² "Ueber die Zellen der Cyanophyceen," Bot. Zeit., No. 38, 1892, and No. 15, 1893.

³ "Ueber die Organization der Phycochromaceenzellen," Bot. Zeit., 1893, p. 73.

⁴ "Beiträge zur Kenntniss des Baues des Cyanophyceen-Protoplasta," Jahrb. für Wisa. Bot., Vol. XXV, p. 511.

The second class of granules, which he identifies with the "Schleimkugeln" of Schmitz, are found in the immediate vicinity of the "central body," but never *in* this structure, and rarely only in the chromatophore. They are identical with the "nucleolus," the "central substance," and the "red" granules of earlier observers, but their significance is unknown to Palla. He calls attention to the fact that in the living cell they are stained with methylene blue, which leaves unaffected peripherally placed granules, formed of the "cyanophycin." The chromatophore has a vesiculated structure, and the vesicular walls are free from colouring matter, which is connected with numerous small granules placed in (?) the vesicles.

In 1894 Fischer¹ reiterated his objections to Bütschli's results, basing these upon more extended studies of the structure of Bacteria.

Nadson's observations were carried out upon a large number of forms fixed and stained in various ways. He found that the coloured peripheral zone of the cell is not a chromatophore but that it is protoplasm containing phycochrome. This peripheral layer is vesiculated in the sense of Bütschli's "Wabenbau." The vesicles are filled with a plasmatic substance which is physically rather than chemically distinguishable from that forming the walls of the vesicles. Chlorophyll and phycocyan are, however, only in the walls. The central body is sharply differentiated from the coloured zone and contains vesicles, but these are less readily observable than those found in the peripheral layer, and they are filled with a strongly stainable substance. Two kinds of granules are present. Of these, one consists of those called "red" by Bütschli, or the "Schleimkugeln" of Palla and Schmidt, and according to Nadson are composed of a substance closely corresponding to chromatin. Such granules occur chiefly in the central body and in the walls of the vesicles, and their number in a cell varies very much, but whether many or few are present the cell is equally capable of division. The second kind of granules, called by Nadson, the reserve granules, correspond to the "cyanophycin" granules of Borzi and Palla, and are composed of a substance comparable to the starch of the Chlorophyceæ. They occur only in the coloured zone, particularly in the neighbourhood of the transverse walls. They colour with hæmatoxylin blue-violet like the protoplasm of the peripheral layer.

Nadson found a third kind of granules, probably of a plasmatic

¹ "Untersuchungen über Bakterien," Jahrbuch für Wiss. Bot., Vol. XXVII, 1894.

² "Ueber den Bau des Cyanophyceen-Protoplastes," (In Russian with resumé in German), Scripta Botanica, Vol. IV, St. Petersburg, 1895. I have not, unfortunately, access to this publication and have had to rely on the reference given in Botanischer Centralbl. Vol. LXIII, p. 238.

nature, in the nodal points of the protoplasm in the peripheral zone. In *Merismopedia* and *Aphanocapsa* the "chromatin" granules in division arrange themselves in the form of a figure 8, which after division falls into two circles.

The central body, in Nadson's view, is similar in many respects to the nucleus of higher organisms, and doubtless corresponds to the same. It may be regarded as the forerunner of the latter, but he doubts if all cell nuclei are derived from it.

Macallum¹ found that the stainable granules in the central body do not dissolve when submitted to artificial gastric digestion, but they disappear after treatment with a solution of potassic hydrate of 0.1 per cent. strength for twenty-four hours. This treatment also deprives the central body of its capacity of fixing colours in itself. These facts were held to indicate that a nuclein-like substance is present in the central body and in its granules, and this view was confirmed by the demonstration of the presence, in these parts, of "masked" iron. The "cyanophycin" or "reserve" granules, which stain specially with picrocarmine, may not contain "masked" iron and dissolve readily in dilute solutions of hydrochloric acid. The substance forming these granules was found to be unlike that constituting the granules of the central body, which was held to be in many respects like chromatin.

Bütschli's² third contribution on the structure of the Cyanophyceæ appeared in the next year and in this he defends the views which he advanced in his earlier publications on the subject, and criticizes the views and observations of Deinema, Hieronymus, Palla, Nadson and Fischer. In answer to the latter's objections he denies that the peripheral layer in the cells of the Cyanophyceæ and Bacteria in his preparations is due to plasmolysis and shows that in some at least of the blue-green forms the existence of a peripheral zone and a central body can be determined in the living cell. In these forms, and especially in some *Oscillaria*, the two parts may be set free through rupture of the cell membranes, and then one can distinguish both parts as very distinct. Bütschli strives particularly to show that the vesiculated (Waben) type of structure prevails in both parts and in the two classes of organisms and for this purpose gives photographs of living forms in which this structure was distinctly demonstrated. He found also that the colouring matter of the peripheral zone appeared to be dissolved in the sub-

¹ "On the Distribution of Assimilated Iron Compounds, other than Hæmoglobin and Hæmatins, in Animal or Vegetable Cells," Quart. Journ. Micro. Sci., Vol. XXXVIII, p. 175, 1895.

² "Weitere Ausführungen über den Bau der Cyanophyceen und Bacterien," Leipzig, 1896.

stance forming the walls of the vesicles (Waben) and not in the contents of the latter.

Amongst the points dealt with by Bütschli were those relating to the nature of the granules and to the homology of the central body. As in his earlier observations he found two kinds of granules; one staining red-violet with Delafield's hæmatoxylin and called on this account "red" granules; the other, in *Oscillaria* usually adjacent to the transverse septa and consequently in the peripheral layer, unaffected by hæmatoxylin but coloured red with eosin and called, after Nadson, "reserve" granules, or, after Borzi, "cyanophycin" granules. The "red" granules are, at times, as Hieronymus and Palla found, hollow bodies. They occur in both parts of the cell but they are chiefly found on or in the outer portions of the central body imbedded in the nodal points of its vesicles. This observation is opposed to the views of Zacharias and Palla, who maintain that the central body is free from granules. The substance forming them Bütschli believes to be chromatin, and the evidence in support of this view he finds in the staining properties of the granules. Hæmatoxylin usually does not stain chromatin red or red-violet, the exceptions being the granules formed of this substance in the nuclei of *Euglena* and Diatoms, but the granules in the cytoplasm of the latter and in some Protozoa give a similar red stain, a fact which tells against "red" granules in Cyanophyceæ being considered as composed of chromatin. Bütschli, however, found that in the living forms the cytoplasmic granules which stain red with hæmatoxylin colour red with methylene blue, but the nuclear granules in *Euglena* and Diatoms are rendered blue with this reagent. Now Lauterborn discovered that in living *Oscillaria* methylene blue also gives a blue colour to the granules which, in hardened preparations, stain red-violet with hæmatoxylin. These facts, Bütschli thinks, indicate that the "red" granules are formed of chromatin.

Bütschli doubts if the "reserve" granules are formed of a carbohydrate, but he believes that the action of iodine solutions on material which has been kept for a long time in alcohol points to the presence of glycogen in the Cyanophyceæ.

The central body Bütschli holds to be a nucleus. The objections to such a view he deals with in detail and points out that the absence of indirect division occurs also in the macro-nuclei of Infusoria, in nuclei of *Amœba*, Dinoflagellata and *Euglena*, while he urges in answer to Zacharias, who could find no nuclein in the central body either during rest or division of the latter, that this does not diminish the suspicion concerning the poverty of our micro-chemical methods

Zacharias¹ confirms his earlier observations, except as to the presence of nuclein in the central body, of which he is doubtful. He regards the central body as differing in important points from the nucleus of other organisms.

Fischer, in his recent publication, gives the results of a more extended series of observations on the structure of the Cyanophyceæ and Bacteria. His first statement is a withdrawal of the view which he held that the "central body" of Bütschli arose through plasmolytic contraction of the contents of the cell, brought about by the hardening reagents used. He finds, however, that Bütschli's description of the granules is far from exact. In many of the forms hæmatoxylin colours some of the granules blue, others red, in other forms all the granules may be coloured blue, while sometimes again the granules may be stained red only. In Bütschli's opinion the reserve granules, the "cyanophycin" granules of Palla, do not stain with hæmatoxylin and, therefore, the granules which colour blue with this dye, do not, according to Fischer, come into Bütschli's classification of the granules. Fischer also found that all the granules stain blue with hæmatoxylin after treatment with soda solutions. On the other hand the "cyanophycin" granules in *Oscillaria tenuis*, after fixation with alcoholic iodine solutions, do not stain with hæmatoxylin, but they will do so if hardened with a four per cent. solution of formol. The granules do not disappear after artificial digestion (in pepsin and hydrochloric acid), nor on treatment with a ten per cent. soda solution. From all these facts he concludes that the differences in the staining power of the granules are due, not to differences in chemical composition of the granules, but to their physical properties, and that the tests upon which Bütschli relied to show that the "red" granules are composed of chromatin are valueless. He regards the substance entering into the composition of all the granules as an assimilation product or a reserve material.

In regard to Palla's contention that granules do not occur in the "central body," Fischer found them in this organ as well as in its periphery. None were observed in what he calls the chromatophore, the zone of coloured protoplasm surrounding the "central body." Granules exist outside this zone and, particularly, adjacent to the transverse septa, a fact which leads Fischer to believe in the existence of a plasma zone outside the chromatophore. The independent existence of the latter organ he claims is shown by the results of treatment with hydrofluoric

¹ "On the Cells of the Cyanophyceæ," Report of the British Association, Liverpool Meeting, 1896, p. 1021.

² "Untersuchungen über den Bau der Cyanophyceen und Bacterien," Jena, 1897.

acid, which dissolves everything else in the cell. Through the chromatophore stretch radial strands of protoplasm from the central body to the plasma zone on the inner wall of the cell. In pepsin and hydrochloric acid the volume of the contents is greatly decreased, but this is not due to digestion of the peripheral portions chiefly and of the central body partly as Zacharias and others believed, but to a contraction which he terms enzymatic and which can be demonstrated in *Spirogyra* also. In the shrunken parts the peripheral as well as the central parts are present. Digestion experiments, therefore, give no certain conclusions as to the special nature of the central body. The latter is in his view simply a portion of the protoplasm enclosed by the chromatophore and containing assimilation products. There is nothing to indicate that it is a nucleus or the homologue of a nucleus. Nor is there any other organ which may be held to represent a nucleus.

II—METHODS OF STUDY AND MATERIAL EMPLOYED.

By hardening and staining highly organized animal and vegetable cells in the way that cytologists usually employ, one may obtain preparations which readily reveal the structure of these elements, but the employment of these and other simple methods in the case of the Cyanophyceæ are not at all as fruitful in results. One finds in such little more than one can recognize in the living cells. It is with such and similarly meagre methods that the majority of investigators in this field of research have contented themselves when the apparently indifferenced character of the cells in these organisms should have suggested the employment of a multiplicity of methods. It is only in this way that one may obtain results which permit a generalization concerning the structure of these forms.

The Cyanophyceæ respond very sensitively to the conditions to which they are subjected. This fact also has been overlooked or not suspected. In a culture of them twenty-four hours will make a complete change in some of the more important features of their cells, and at the same time different parts of the same culture, not more than a few centimetres distant from each other, may present specimens of the same species in which the cell contents are markedly unlike. It is to this fact that we may attribute discrepancies in the descriptions, by the various observers, of the structure of these organisms. To illustrate particularly how important this point is, I may refer to Palla's observations on *Glæotricha pisum*. In single cells of this form he found large vacuoles, and more

than one central body. The material which he used must have been in a pathological condition, for it is only when *Cylindrospermum majus* is ill-nourished that any one of its cells possesses similar vacuoles and a plurality of central bodies.

It is obvious, therefore, that a study of the cells of the Cyanophyceae involves the employment of a large number of methods of manipulation, and a regard for material in all conditions of nutrition. The latter requisite also entails a careful attention to the various conditions in which the organisms are usually found, and an acquaintance with the modifications that a varied environment brings about. These conditions and these modifications are, at present, not fully known, but what we do know helps in determining what is the typical structure of the cell in this class.

I have, therefore, in all cases used material from each species which was found to be in an active state of growth. This is a safeguard of great value. The material which one may collect from any locality may be in the resting stage, a stage in which also, perhaps, changes analogous to those of involution in Bacteria may manifest themselves. The only material which one can confidently regard as normal is that which grows in the laboratory, and which can be examined from hour to hour, the change in the volume of the material being an indication of its active growth.

The species¹ used in these studies were *Microcoleus terrestris* Desmazières (*M. vaginatus* Gomont), *Oscillaria Froehlichii* Kützing (*O. limosa* Agardh), *Oscillaria natans* Kützing (*O. tenuis* Agardh), *Oscillaria tenerrima* Kützing (*O. amphibia* Agardh), *Oscillaria princeps* Vaucher (*O. maxima* Kützing), *Cylindrospermum majus* Kützing, *Tolypothrix tenuis* Kützing, *Tolypothrix rupestris* Kützing, *Nostoc commune*, *Rivularia* sp., *Glaucocapsa polyderrmatica*, *Lyngbia* sp., *Scytonema* sp.

The hardening reagents employed were: corrosive sublimate in saturated aqueous solution, in saturated alcoholic solution, and in conjunction with picric acid, picric acid in saturated solution, Flemming's chrom-osmio-acetic mixture in strong and weak solution, osmic acid in one per cent. solution, alcohol absolute and of ninety-five per cent. strength. I have used also aqueous and alcoholic solutions of iodine. The best preparations were made with the picric acid and corrosive

¹ Gomont, "Monographie des Oscillariées, (Nostocacées homocystées)," *Annales des Sciences Nat., 7ième Série, Botanique*, Tome XV, p. 263, Tome XVI, p. 91.

Also, Bornet and Flahaut, "Revision des Nostocacées heterocystées contenus dans les principaux herbiers de France," *Annales des Sciences Nat., Botanique, 7ième série*, Vols. III, IV, V and VII.

sublimate reagents. The former was allowed to act for forty-eight hours, the material was then placed in seventy per cent. alcohol, which was replaced by a fresh quantity daily for a week, after which it was transferred to alcohol of ninety-five per cent. strength. The corrosive sublimate solutions were allowed to act for an hour only, and the subsequent treatment with alcohol was the same as in the case of the picric acid material. Material hardened in alcohol was used only for the iron and phosphorus reactions.

The staining reagents found to be serviceable were Ehrlich's and Delafield's hæmatoxylin solutions, Czokor's alum cochineal, safranin, eosin, picrocarmine and methylene blue. Material hardened with alcohol, picric acid or corrosive sublimate gave very valuable preparations when stained with picrocarmine solution for twenty-four hours, then for an hour with a dilute solution of hæmatoxylin. The picrocarmine specially selects the granules which Borzi and Palla term "cyanophycin," while the hæmatoxylin thus employed stains the "central body" and its granules particularly and the peripheral protoplasmic zone less distinctly. The "central body" in corrosive sublimate preparations is less clearly differentiated from the peripheral zone.

There is one advantage in the use of picric acid which does away with the necessity of resorting to transsections of the trichomes, for the reagent seems to have some solvent or disintegrating effect on the substance of the membranous sheath and of the transverse septa, the trichomes breaking up, under the slightest pressure of the cover-glass, into the separate cells which very frequently then are seen by their flat or end faces.

The method of obtaining the reactions for "masked" iron in the Cyanophyceæ I have fully described elsewhere.¹ The iron, liberated by sulphuric acid alcohol, as indicated, was converted into Prussian blue, the trichomes were then stained with a picrocarmine solution for twenty-four hours when the cyanophycin granules acquired a deep red colour which contrasts markedly with the Prussian-blue tint of the ironholding granules. (Fig. 14). Instead of acid alcohol strong solutions of hydrogen peroxide which contained traces of sulphuric acid were frequently used to liberate the masked iron.

The demonstration and localization of organic phosphorus were effected in the manner which I have indicated elsewhere,² but I may here briefly

¹ "The Distribution of Assimilated Iron Compounds, other than Hæmoglobins and Hæmatins, in Animal and Vegetable Cells," *Quart. Jour. Micro. Sci.*, Vol. XXXVIII, p. 175, 1895.

² "On the Detection and Localization of Phosphorus in Animal and Vegetable Tissues," *Proceedings Roy. Soc.*, Vol. LXIII, p. 467, 1898.

describe the method used. The material, after thorough fixation with alcohol, was washed free from the latter with distilled water, then put in the nitric-molybdate solution for three to six hours at a temperature of 35° C., and finally, after quickly washing in water, treated for a few minutes with a one per cent. solution of phenylhydrazin hydrochloride, which converts the phospho-molybdate into a bluish-green compound, this colour reaction thus indicating the original distribution of the organic phosphorus. To provide against the phosphorus demonstrated being that of lecithin, the material was extracted with hot alcohol in a Soxhlet apparatus for five hours.

The other methods resorted to included: digestion of fresh material with artificial gastric juice and with alkaline solutions of trypsin, staining of fresh material for twenty-four hours with acetic-methyl green and subsequent fixation by saturated solutions of picric acid and the treatment of fresh material with solutions of Ehrlich's hæmatoxylin. The digested material was similarly stained and all the preparations were mounted in glycerine.

III.—THE LIVING CELL IN THE CYANOPHYCEÆ.

In the larger species of Cyanophyce the cell substance readily reveals the existence of two zones, one, peripheral, coloured blue-green, the other, central, containing granules and, but for the shimmer of blue-green of the outer zone through which it is seen, colourless. In Fig. 1 the division of the cytoplasm is very clearly indicated. The smaller the cell the greater is the difficulty with which the central uncoloured zone is observed and in those species in which the cell is long and narrow, e.g., *Microcoleus terrestris* or *Oscillaria tenerrima*, the central zone cannot be seen, it being of such minute dimensions that its presence is masked by the outer zone.

Whenever it can be distinctly seen the central zone, or, as it is usually called, the central body, is found to contain granules which vary in size and numbers, but these most frequently appear in the outer portion of the central body and in some cases the inner portion of the latter may be completely free from them. Apart from the granules the central body is uniform in structure, appearing to possess a vesiculated structure which comes out quite distinctly in *Oscillaria princeps*. The vesicles in the latter are not closely aggregated and are separated from one another by coarse trabeculæ of a colourless, hyaline, plasma-like substance in which excessively minute, punctiform appearances suggest a granulation

of quite another order than that already referred to. In this species the demarcation of the central body from the coloured, peripheral zone is not sharp and definite, the one passing gradually into the other.

In the peripheral, coloured zone the details are more difficult of observation. One can indeed see granules, but their relation and the distinction between these and portions of the protoplasmic trabeculae are difficult to determine in the fresh condition. If a fresh specimen of *O. princeps*, *O. Froehlichii* or *O. natans* is observed under the apochromatic 1.5 mm. and compensation ocular 8 or 12 with best light (that from a white cloud), one can see in the very uppermost plane of the cell the membrane studded with refracting points which, when the tube of the microscope is slowly lowered, resemble granules, but at the sides and ends of the cells they appear as elongated elements which radiate from the central body. The refracting points are, therefore, really the terminals of protoplasmic trabeculae which connect the central body with the membrane. At certain positions of the objective these trabeculae appear colourless, but at others they have a green tinge which may be due to the reflection of green from the colouring matter. Whether the latter is in the substance of the trabeculae or in the fluid held between them it is impossible to say. There are, however, as will be seen afterwards in the description of the structure of the hardened cell, indications that the blue-green mixture is held in the cavities between the trabeculae. On the other hand I have never seen granules holding chlorophyll such as Hieronymus describes. Of the occurrence of a special chromatophore in any form there seems to be not the slightest indication found in the living cell. I regard the granules, which to Palla appeared to contain a mixture of chlorophyll and phycocyan, and the granules observed by Hieronymus as optical transsections of the protoplasmic trabeculae which extend from the central body to the cell wall. I have never found these trabeculae formed of other than homogeneous substance and can, therefore, not support the view of Hieronymus that coloured granules are imbedded in numerous parallel fibres which run in a spiral fashion in the coloured zone about the long axis of the cell. I have never observed what Palla confidently asserts, namely, that the pigment granules are arranged in rows.

In the outer or peripheral zone there occurs a large number of granules which do not contain any colouring matter, although they may at times be so situated in the coloured zone as to appear coloured, this being due to their reflecting the blue-green of the surrounding cytoplasm. In the spores of *Cylindrospermum majus* large granules imbedded in the substance of the peripheral zone, and by their presence forming bays in

central body, appear blue-green, but that they are uncoloured can be determined readily by causing the spores to burst, when the granules, becoming free, appear unpigmented. These, to a certain extent, correspond, as will be shewn below, to the "cyanophycin" granules of Palla and Borzi. "Cyanophycin" granules are usually abundant in the *Oscillaria*, being found in a row at each end of the cell adjacent to the transverse septum, but in *Microcoleus terrestris*, in *Tolypothrix*, *Scytonema* and *Lyngbia*, they are distributed throughout the substance of the peripheral zone.

IV.—FIXED PREPARATIONS OF CYANOPHYCEÆ.

When trichomes of *Oscillaria Froehlichii* have been hardened in picric acid for several days and stained with picrocarmine and hæmatoxylin, the preparations contain isolated elements like those represented in Figs. 2 and 3. Similar preparations are obtainable from this form when corrosive sublimate is used as a fixative agent, but the vesiculation of the cytoplasm, while distinct, is less clearly demonstrated than in picric acid preparations. Like preparations may be obtained with Flemming's fluid.

The cytoplasm has the structure which Bütschli claims obtains in it. The character of the vesiculation is, however, not what he describes nor does every trichome even in a picric acid preparation present a similar vesiculation. In all, on the other hand, the vesiculation of the central body appears of a finer character than that in the peripheral zone. When the spaces in the cytoplasm are, as is the case in some trichomes, very minute it would be difficult to determine whether vesiculation obtains, were it not that some threads show all the stages between this condition and that in which the vesiculation is distinctly pronounced. In the latter the walls separating the vesicular cavities have a membranous appearance with an indefinite character to their borders.

Owing to the more minute character of the vesicles in the central body, the latter has a compact appearance, and as it stains with hæmatoxylin and other dyes somewhat deeply and uniformly it is thereby brought out sharply in contrast with the cytoplasm of the peripheral zone. There is usually not a distinct line of demarcation between them, but the vesiculation of the central body may pass almost abruptly into that of the peripheral zone. The vesicles in the latter are elongated, tending to extend from the central body to the membrane of the cell. In consequence of the peripheral layer being thicker at the sides than it is

adjacent to the transverse septa, the membranous bands separating the vesicles in the neighbourhood of the septa are shorter than elsewhere. The bands terminate in a thin delicate layer closely applied to the cell membrane, which can be seen with difficulty and is most evident when, as in *Oscillaria Froehlichii*, it is, as in that part adjacent to the transverse septa, rich in granules of the "cyanophycin" class. The latter are usually at "nodal" points of the layer and membranous bands terminating in it. The layer may sometimes be distinctly seen in preparations of *Oscillaria Froehlichii* which have been hardened in alcohol and stained with hæmatoxylin. In these the cytoplasm has shrunk away from the membrane at one side of the cell in many trichomes and in this case the thin hyaline layer, stained somewhat more deeply than the enclosed cytoplasm, appears sharply contrasted with the latter.

In *Oscillaria natans*, *Tolypothrix tenuis*, *Scytonema* sp., and in the vegetative cells of *Cylindrospermum majus* the structure of the cytoplasm in the fixed condition is the same as in *Oscillaria Froehlichii*. In these forms, however, the cells are much smaller and consequently the vesiculation, which may be clearly observed in the larger form, is only rarely well seen. On the other hand the distinction in the cytoplasm of a central body and a peripheral zone is quite as readily marked as in the large forms of *Oscillaria*. The central body also stains in hæmatoxylin more deeply than the peripheral cytoplasm, into which it is continued without any sharp transitional changes in staining or structure.

In *Microcoleus terrestris* owing to the narrow transverse diameter of the trichomes the differentiation of the cytoplasm into a central body and a peripheral zone is not perceptible. There is a difference between the most centrally placed cytoplasm and that of the periphery, but where one begins and the other ends it is impossible to say. The most centrally placed part stains more deeply in hæmatoxylin than does the peripheral part and at the same time appears denser. Vesiculation in both parts has been often observed but there is no distinction in the size of the vesicles of the two parts. In the central part more of cytoplasmic substance appears to surround each vesicle when the vesiculation is distinct. In *Oscillaria tenerrima* there is apparently no distinction between central part and peripheral layer, except in the deeper staining of the more central cytoplasm. Vesiculation was not observed in this form. (Fig. 16).

These facts support on the whole Bütschli's claim that there are two parts, a central and a peripheral, and that the character of the latter is different from that of the former. Bütschli's view that the central

body is the homologue of a nucleus is another question which must be determined, not only on structural but also on micro-chemical grounds.

There is not, as already pointed out, a distinct line of demarcation between the central body and the peripheral cytoplasm. There is also nothing to indicate the occurrence of a membrane about the central body. Further, no elements can be found in the central body which correspond to the chromatin nodules or "nucleolar" structures, although as will be shown below, granules of a chromatin-like substance obtain in the more peripheral portions of the central body, but rarely in the central portions of the latter. It must also be pointed out that no observer, with the exception of Scott, has described any structures in the Cyanophyceæ which correspond to those found during mitosis in the higher organisms. This latter fact, perhaps, taken by itself does not count much against the view that the central body is the homologue of a nucleus, for, as Bütschli points out, the macro-nucleus of Infusoria never exhibits mitotic division, but, taken in conjunction with the other facts regarding the structure of the central body, renders it exceedingly hypothetical that the central body corresponds to the nucleus of the higher organisms.

From the micro-chemical point of view the evidence for Bütschli's view would appear to be somewhat stronger. I have already pointed out elsewhere¹ that the substance of the central body gives indications of the presence of a compound containing "masked" iron. Further observations on a number of the larger Cyanophyceæ confirm this. Trichomes of *Oscillaria Froehlichii*, *Tolypothrix tenuis*, *Cylindrospermum majus*, hardened in alcohol, mounted on a slide in a mixture of glycerine and ammonium hydrogen sulphide and kept in a temperature of about 60°C, gave, after some days, preparations in which one observed a somewhat dark-green tint in the central body. Very often when the preparation had not been a success the result was due to the slow penetration of the sulphide which can only act successfully when the reagent attacking the iron-holding body can be quickly renewed. The iron reaction, when it is obtained in this way, is distinct and does not occur in the peripheral zone. The iron reaction may, however, be obtained more readily in another way. If the trichomes, hardened in alcohol, are placed for two to five hours at 35° C. in sulphuric acid alcohol (acid 4 vols., alcohol, ninety-five per cent., 100 vols.), and then after washing in ninety-five per cent. alcohol, are treated with the acid ferrocyanide mixture (potassium ferrocyanide, 1.5 per cent. solution, hydrochloric acid,

¹ "On the Distribution of Assimilated Iron Compounds, other than Hæmoglobins and Hæmatins, in Animal and Vegetable Cells," Quart. Journ. Micro. Science, Vol. XXXVIII, p. 175.

0.5 per cent., equal volumes) for five minutes to give the Prussian-blue reaction, or with aqueous hæmatoxylin (0.5 per cent. solution) for a few minutes, preparations are obtained which show quite distinctly that the central body contains a substance in which "masked" iron is held. At the same time one species of the granules also may show the presence of "masked" iron and this may be observed when the glycerine sulphide method is used. The Prussian-blue reaction exhibited by the central body is a uniform one and the depth of the blue colour depends on the thickness of the cell but it is unmistakable in every case. The bluish-black given by the hæmatoxylin is similarly marked. It is to be noticed, however, in all these cases that the blue of the Prussian-blue reaction and the bluish-black of the hæmatoxylin are not limited to the central body, for a lighter blue or a faint hæmatoxylin reaction may be found in that part of the peripheral zone next to the central body, and very often in such preparations it is not possible to say where the line of separation is. This is the case sometimes in *Oscillaria Froehlichii* and if the cell discs are seen from their flat surface the blue colour is marked in the centre but gradually diminishes towards the periphery and is obscure or absent in the outer part of the peripheral zone. A similar distribution of the "masked" iron in the peripheral zone is shown by the glycerine-sulphide method, but the reaction is not as decisive for minute quantities of iron.

There can, therefore, be no doubt that the central body specially, and a portion of the peripheral zone immediately about it in a very much less degree, contain "masked" iron.

The reaction for organic phosphorus is not less distinct. Treatment of trichomes, hardened in alcohol, with a nitric acid solution of ammonium molybdate for several hours at a temperature not exceeding 35° C., then with dilute nitric acid for a few minutes and afterwards with a two per cent. freshly prepared solution of phenylhydrazin hydrochloride for from three to five minutes, gave definite indications of the presence of phosphorus in the production of a dull greenish-blue reaction in the central body specially and only faintly in the cytoplasm of the peripheral zone. Certain granules also give the reaction deeply as in the case of the iron reaction. The reaction in the central body is, as stated, a marked one, and it is uniform, shading at the margins of the central body into the feeble reaction of the peripheral zone. That the reaction is due to organic phosphorus and not to inorganic compounds of this element is indicated by the fact that the nitric-molybdate reagent brings out the full reaction after two hours at the earliest, and then only gradually.

The presence of "masked" iron and organic phosphorus in the central
[60]

body is at least an indication of the occurrence therein of an iron-holding nuclein, like, in some respects at least, the chromatin of the nuclei of higher organisms. This conclusion is supported by the results of the action of artificial digestive reagents in these organisms.

When fresh trichomes of *Oscillaria*, *Tolypothrix*, *Scytonema* and *Microcoleus* were digested with artificial gastric juice, made by adding a quantity of a strong glycerine extract of the mucosa of the gastric fundus of the pig to hydrochloric acid of 0.2 per cent. strength, the preparations at the end of forty-eight, seventy-two and ninety-six hours showed certain changes which were due to the digestive reaction. Zacharias found that the peripheral zone is in great part removed, while a portion of the central body disappears leaving two substances behind, one of the nature of plastin, the other having a nuclein-like character and receiving from him the name, "central substance." Fischer, on the other hand, denies that the digestive reagent has any such effect and that the diminution of the volume of the contents is due to a contracting action of the digestive ferment in the presence of hydrochloric acid, an action which he terms enzymatic. Fischer's view is incorrect for when one studies at some length the digestive action of artificial gastric juice on the cells in Cyanophyceæ, not only is there a diminution in the volume of the cell contents but there is also a disappearance of a portion of its constituents. The peripheral zone is perhaps most affected but a portion of it resists digestion, and after treatment with weak potash solution (0.3 per cent.) remains. This latter substance is undoubtedly plastin. The central body also is affected, but at first sight apparently less so than the peripheral zone. It is rarely diminished in size and it has, as Zacharias points out, specially after treatment with alcohol and ether, a nuclein-like lustre. When, however, the preparation, after prolonged treatment with alcohol and ether, is stained in a good alum-hæmatoxylin solution (Ehrlich's or Delafield's) the central body, instead of appearing homogeneous or appearing uniformly stained as in good fixed preparations, gives in the majority of cases a coarse reticular appearance in which the trabeculæ are usually deeply stained. (Fig. 8).

The stainable portion of the central body, the "central substance" of Zacharias, is soluble in weak alkalies, as may be proved by placing trichomes, acted on by gastric juice for forty-eight hours, in a 0.3 per cent. solution of potash for six or seven hours, when subsequent treatment with ether, alcohol, and Ehrlich's hæmatoxylin fails to indicate the occurrence of a stainable substance like that referred to. What remains of the central body and of the peripheral zone stains, but there is no differentiation both parts staining feebly but uniformly. This indicates

that what remains in the central body as well as in the peripheral zone is formed of plastin only.

These facts show quite clearly that artificial gastric juice does dissolve a portion of each part of the cell in the Cyanophyceæ. That which is left in the peripheral zone is largely, if not wholly, composed of plastin while a part of what is left in the central body appears to have the character of a nuclein compound. The concentrated character of the substance left in the central body and its coarsely reticular appearance can only be explained on the postulate that the reagent has removed material from the central body. What contributed to the view of Fischer that the digestive fluid exercises only a contracting, not a dissolving action, was the assumption that nothing appeared to be removed from the cell acted on by the digestive fluid. There is one feature in all digestive experiments on the Cyanophyceæ which ought to be borne in mind. The membranes in the Cyanophyceæ have a colloid character and as such they do not readily permit the diffusion through them of colloids in solution. Pepsin is much more a colloid than a crystalloid and consequently it will not penetrate the membranes in the Cyanophyceæ with the same facility as hydrochloric acid and, therefore, a slighter effect is produced at the end of twenty-four hours than is obtained when naked cytoplasmic masses, like the cells in higher organisms, are treated with gastric juice. This would account in part for the results obtained by Fischer.

There can be, therefore, very little doubt that a chromatin-like substance is present in the central body of cell of Cyanophyceæ. It is much less in amount than the chromatin of the smallest nucleus in a highly organized cell, and it does not appear to vary in amount in the various cells of a trichome nor in those of a preparation of filaments. In other words, this chromatin-like substance appears to be fixed and unchanging in amount in whatever condition the cells may be. This chromatin-like substance never appears to enter any condition resembling, in the remotest degree, that of mitosis and whenever the cell divides this substance is distributed as it is in the resting cell, that is, uniformly throughout the central body. It is impossible, then, to regard the central body, with Bütschli, as the homologue of a nucleus. From its structure and its chemical character it is rather to be regarded, as Fischer claims, as a more active portion of the cytoplasm.

When trichomes of *Oscillaria*, *Tolypothrix*, *Scytonema*, *Microcoleus terrestris*, are digested for twenty-four or forty-eight hours with artificial gastric juice and then stained for twenty-four hours with picrocarmine, what remains of the central body is coloured red and at times the peri-

pheral zone is given a green stain. A similar result has sometimes been obtained with Ehrlich's hæmatoxylin in dilute solution, but in this case the central body takes a blue violet colour. When alcohol and ether were employed after the gastric juice the green reaction was not obtained, and this was the case also when the digested trichomes were treated with weak alkaline solutions, this showing that the substance which stains green is soluble in alcohol and alkalis. Far oftener, however, another condition was observed. Digestion of trichomes for two or three days resulted in the production of somewhat irregular masses of brownish material placed frequently adjacent to the transverse septa. In *Oscillaria tenerrima* there may usually be only one such mass in each cell, being flattened and situated next to either the lateral membrane or one of transverse septa. In *Microcoleus terrestris* the corresponding brown substance is in the form of granules, many of which are distributed through the cell. In all cases the substance forming these masses is soluble in alcohol and in weak alkalis. The masses may also take, after a prolonged stay in picocarmine solutions, a dull green or bright green colour. Their solubility in alcohol and weak alkaline solutions, their absence when the whole of the peripheral layer stains green with picocarmine, and their reactions with the latter dye, appear to indicate that they are derived from a constituent uniformly distributed throughout the peripheral zone in the living cell. The green reaction under the influence of picocarmine indicates a chromogenic character and it is probably a derivative, through digestive action, of one of the constituents which form the "blue-green" of the peripheral zone.

The occurrence of such a pigment, if it is derived from the colouring matter of the cell, insoluble as it is in water, renders it probable that the colouring matter during life is dissolved for the most part in the cavities of the vesicles of the peripheral zone. If it were dissolved in the cytoplasmic framework it would be difficult to explain its occurrence, after digestion, in masses.

The question of the occurrence of a chromatophore has been dealt with in various ways by the majority of investigators of the structure of the cell in Cyanophyceæ. Whatever is meant by the term chromatophore, it is necessary to point out that such an organ as it obtains in the more highly specialized Algæ can never be found in Cyanophyceæ. All who have claimed that a chromatophore is present in the Cyanophyceæ are not agreed as to its character. According to Deinega it is a perforated plate, the trabeculæ of which carry the colouring matter and run parallel, in the greater number of cases, with the trichome. In Zukal's view the coloured, peripheral zone is the chromatophore which he claims is finely

punctated, while Hieronymus maintains that it is of fibrillar structure and on it are arranged fine granules which contain the chlorophyll, the phycocyan being dissolved in the enchylema. Palla, on the other hand, defines, without further reference, the coloured peripheral layer as the chromatophore, which Nadson does not accept, but according to Fischer the chromatophore is an independent organ which is perforated to allow the passage of protoplasmic strands from the central body to the plasma zone outside the chromatophore. Fischer in his figures of stained preparations of *Oscillaria Froehlichii* illustrates such a radial disposition of protoplasmic strands from the central body but there is in them no indication of the existence of a chromatophore and the only evidence which he advances for believing that the latter exists is the result of the action of strong hydrofluoric acid on specimens of *Oscillaria princeps*, which dissolves out the central body, leaving a ring of material undissolved corresponding in position to the coloured zone of the cell. I have tried the action of hydrofluoric acid on specimens of *Oscillaria Froehlichii* and have not been successful in obtaining the results that Fischer obtained. Giving full importance, however, to his observations on this point one cannot but at the same time question whether the structure observed after the action of such a drastic reagent as strong hydrofluoric acid, is not simply an artifact. As he describes and illustrates it, it is a compact solid body without structure, and this is a suspicious fact, for, in his view, the protoplasmic strands which penetrate it are numerous. Observing also his illustrations of transsections of stained *Oscillaria Froehlichii* one must ask also how such an organ could be accommodated in the peripheral zone, the greater part of the space of which is taken up by the protoplasmic strands.

There is, as I have already pointed out, no evidence for the existence of a specially organized chromatophore to be found in the living cell. When a cell of *Oscillaria princeps* in the living state is ruptured the substance of the peripheral zone is found to have a much more fluid character than that of the central body. This shows that, whatever the chromatophoric substance may be, it is not distinct from the substance of the peripheral zone, nor has it the physical character of a chromatophore as found in the green Algæ.

Considering the fact that there is in the Cyanophycæ no nucleus it is reasonable to believe that the other structures in the cell are as little differentiated, and therefore, one should not expect to find in these forms a highly organized chromatophore. This view is the most consistent, moreover, with all the observations which have been made on the Cyanophycæ.

V.—THE GRANULES IN THE CYANOPHYCEÆ.

The granules, according to Bütschli, Palla and Nadson, are of two kinds, one which stains red-violet with Delafield's hæmatoxylin and situated, according to Palla, on the periphery of the central body, according to Bütschli on the central body or in its more peripheral portions, according to Nadson, in the central body only; the other which stains blue or blue-violet, to be found chiefly adjacent to the transverse septa. The latter class of granules Bütschli claims is not affected by hæmatoxylin staining, and he usually demonstrated their presence with eosin. Zacharias also has described the occurrence of two kinds of granules, and the writer showed that one kind, those which are described by Bütschli as staining red-violet with hæmatoxylin, contain a "masked" iron compound, while the other kind of granules are, as a rule, free from "masked" iron. Hieronymus, Deinega and Fischer, however, claim that there is only one kind of granules present. The first-named observer holds that two kinds of granules appear to be present because of the methods of preparation, but that if fixed cells are treated with ammonia vapour all the granules colour dark blue with hæmatoxylin and further that all dissolve in dilute acids although not equally readily. Fischer, on the other hand, maintains that the granules do not disappear in artificial digestion (with pepsin and hydrochloric acid) and further that, if they are treated with dilute soda solutions, all stain blue with hæmatoxylin. He also finds that in some forms all the granules are stained either blue or red, while in other preparations some of the granules stain blue, the remainder red. He claims that the method of fixation employed greatly varies the capacity of some of the granules, notably those which stain blue with hæmatoxylin, to absorb the staining compound, and he concludes that staining is due to differences, not of chemical properties, but of physical condition produced by the fixing reagent. The granules which Deinega found were soluble in acids and stained specially with picrocarmine.

I have made a lengthy examination of the granules which occur in the Cyanophyceæ and I find that while in some species one kind only may be present, yet in other forms two distinct types of granules occur. Illustrations of these two types may be found in the *Oscillariæ*, in *Tolypothrix*, *Scytonema* and *Microcoleus terrestris*. The best method of demonstrating them is to harden the material in picric acid or corrosive sublimate, or even in alcohol alone, and then stain first with picrocarmine and afterwards with dilute Ehrlich's hæmatoxylin. The latter

reagent gives granules in the peripheral portions of the central body a deep reddish-violet stain and these correspond to the "red" or red-violet granules of Bütschli. These may be, for the present, called the granules of the first type. The granules of the second type stain bright red with picrocarmine and correspond to those which Deinega found and to the granules which, according to Palla and Nadson, stain blue with hæmatoxylin.

The granules of the first type vary very much in size. They are usually situated in the outer sections of the central body although occasionally a single one may be found in the more central portion and in the inner zone of the peripheral layer. The granules, as Hieronymus, Palla and Bütschli found, are, at times, hollow bodies, while to Zacharias their peripheral substance appeared to have a greater density than their centre possessed and the author found that all the larger granules, at least, are formed of an outer zone or coat which takes the hæmatoxylin deeply, with a central cavity containing a fluid substance which is indifferent to staining reagents. Methylene blue deeply stains these granules and in consequence their hollow character in such preparations is not evident. Acetic-methyl green gives them in fresh preparations a stain much deeper than that taken by the central body, while it leaves unaffected the granules of the second type.

The granules of the second type correspond to the "cyanophycin" granules of Borzi and Palla and, as they have a special affinity for picrocarmine, their presence can always be readily demonstrated. In the fresh cells they stain deep blue with Ehrlich's hæmatoxylin. They are in the *Oscillariæ* more abundant adjacent to the transverse septa and they appear to be situated in the thin cytoplasmic layer which is applied to the septum. In picric acid preparations of *Oscillaria Froehlichii* stained with picrocarmine, views of the flat faces of the discs frequently show a somewhat radiate arrangement of the granules, the larger ones of which are found near the junction of the lateral membrane with transverse septa. The granules in such cases are not exactly round, as they appear slightly elongated in the radial direction. In *Microcoleus terrestris*, *Tolypothrix*, *Scytonema* and *Lyngbia*, these granules are distributed throughout what corresponds to the peripheral zone in these forms.

It is not in staining alone that I would base the classification of these granules. A far stronger distinction lies in the chemical reaction of the granules and in the action of digestive fluids upon them.

The granules of the second type very quickly dissolve in hydro-

chloric acid solution of 0.5 per cent. strength, and in very dilute nitric acid and sulphuric acid, while those of the first type are unaffected. As a rule they have no special affinity for iodine over that of the cytoplasm of the peripheral zone. When material, hardened in alcohol, was treated with the nitric-molybdate reagent for several hours and then with a solution of phenylhydrazin hydrochloride, these granules gave no reaction and were consequently not visible, while in the granules of the first type the outer coat, which stains deeply with hæmatoxylin, gave a strong reaction for phosphorus. As this reaction required time to develop, the phosphorus demonstrated must be present in organic form, that is, in the form which characterizes the nucleins and nucleo-proteids.

When material, hardened in alcohol, was treated at 35° C. with sulphuric acid alcohol to set free organic iron, and the iron, thus set free, demonstrated by either the Prussian-blue or the hæmatoxylin method, one found a distinct reaction in the granules of the first type but not in the second. When the granules were large, as represented in Figs. 11-14, the outer coat only gave the reaction, but when a granule was very minute the iron appeared to be uniformly distributed through it. The iron reaction was also obtained in these granules by placing the broken-up trichomes on a slide in a drop of the glycerine-ammonium sulphide mixture and covering the preparation with a cover glass, after which it is placed in a warm oven kept at a temperature of 60° C. for a week, during which the preparation was examined from time to time. The only obstacle to success in these preparations is the presence of the thick membrane or sheath of the trichome which, as already pointed out, prevents a free diffusion of the sulphide into the cells which may consequently not exhibit any reaction, but, in those portions of the trichomes freed from the investing sheath, the reaction comes out quite distinctly in from three to five days. The threads of *Microcoleus terrestris* are most readily freed from their membranes and if granules of the first type are present, which is the case if the form is growing vigorously, they give the dark-green reaction of ferrous sulphide in about three days, and it is distributed as it is found after treatment with acid alcohol, that is, in the outer portions of each granule. No reaction was obtained in granules of the second variety.¹

The iron demonstrated in this case can belong only to the "masked" form, and as such is most usually associated with a nuclein or nucleo-proteid. This and the fact that the granules in question contain organic

¹ An exception must be made in the case of those preparations referred to in my former paper, "Quart. Journ. Micro. Sci.," Vol. XXXVIII, p. 266. As I have never since succeeded in obtaining a similar preparation I have concluded that in that case the granules were not of the normal composition.

phosphorus indicate very decidedly that the substance forming the outer portion of the granules belongs to the nuclein class of compounds. As this same substance has an affinity for hæmatoxylin and acetic-methyl green, it is difficult to resist the conclusion that it is true chromatin.

If, however, trichomes containing these granules are submitted to digestion with artificial gastric juice, the granules in from eighteen to twenty-four hours disappear. The granules of the second type disappear during the first hour. The absence of granules of the first type is not apparent only, as Bütschli claims, who holds that they are still present, although they have lost their capacity to take up stains, but is due to actual solution and disappearance of their substance, for by no method of staining or impregnation can they be brought into view. Bütschli's contention, therefore, is incorrect. It is the recognized property of nucleins to resist gastric digestion and if this is universally true the substance forming the granules cannot be regarded as true nuclein compounds or as a true nucleo-proteid. It must be noted, however, that some forms of nucleic acid are soluble in artificial gastric juice and it is possible that the substance forming the essential portion of the granules in question contains one of these soluble forms. It must be recognized, also, that the substance in question, while possessing many of the characters of chromatin as it is found in highly organized animal and vegetable cells, does not fully represent the latter, and the true representative of chromatin in the Cyanophyceæ is to be found in the substance, holding "masked" iron and organic phosphorus, obtaining in the central body.

What the chemical composition of the granules of the second type is it is not possible to say definitely. The substance forming them has by some been regarded as an isomer of starch, but its capacity for absorbing picrocarmine and staining blue-violet with slightly diluted Ehrlich's hæmatoxylin in the fresh trichome indicates that it is rather related to the proteid class of compounds. This is confirmed to a certain extent by evidence of the presence of organic sulphur in the granules. When preparations of *Oscillaria Froehlichii*, hardened in alcohol, are carefully heated for about ten minutes in a mixture of glycerine and solutions of potash and lead acetate the granules in question exhibit a light brown tint due to the presence of lead sulphide. Except in one instance they have not given any indication of the presence of organic phosphorus and they cannot, therefore, be regarded as formed of a nucleo-proteid compound. They are extractable with boiling water and their substance is not coagulable with heat. On the whole, the facts suggest that they are constituted of a substance which has the characters of one of the

lower proteids. As they are abundant in the rapidly growing and assimilating forms it is possible that their substance represents the first assimilation stage of the proteids synthesized in these organisms. As the granules disappear in trichomes which are not assimilating freely, they would appear to represent the reserve material of the cell, as Nadson and others have claimed.

In *Cylindrospermum majus* only one kind of granules is present and these are usually very large and homogeneous. They resemble the granules of the first type in that they have given evidence of the presence of "masked" iron¹ and also those of the second type in their capacity for absorbing and retaining picrocarmine. I am unable to say whether they contain organic phosphorus, since I have not had at my disposal, during the last two years, any specimens of the organism to determine this point.

In *Nostoc commune* and *Oscillaria tenerrima* only one kind of granules is present. In the latter they are comparatively large and usually hollow in the centre. (Fig. 16).

VI.—THE HETEROCYST.

The development of the heterocyst depends on degenerative changes in the cell which at the outset is free from granules. The first evidence of the change occurs in the disintegration of the central body, which appears to be constituted of a coarse network in which ill-defined granular swellings are seen. This network stains deeply in hæmatoxylin and as the change progresses it fills the whole of the cytoplasm (Fig. 17, two lowest cells). At the same time a large granule or mass develops at one pole of the cell which quickly acquires a marked affinity for picrocarmine (Fig. 15) like that exhibited in the granules of the second type. Very often the cell which is to form a heterocyst is incompletely separated from its neighbour on division, and in the connecting strand of cytoplasm which extends through an opening in the transverse septum, the mass in question develops as a pear-shaped body with its terminal points in the two cells. (Fig 17). This structure has been referred to by Borzi, who believes that it is formed of cyanophycin. When the developing heterocyst is intercalary there may be such a mass at each pole of the cell, but there is only one when the cell is terminal.

In the next stage the structures of the central body dissolve completely

¹ The extent of the reaction for iron obtained is indicated in Fig. 8, Plate 10, "Quart. Journ. Micro. Science," Vol. XXXVIII.

in the cytoplasm which now attains a light greenish-yellow colour. The mass of picrocarmine-staining substance persists, but otherwise the cytoplasm is homogeneous, and it does not stain with hæmatoxylin, picrocarmine, or with any of the aniline dyes. It gives a feeble but uniform reaction for "masked" iron, a slightly more marked reaction for phosphorus and it is not affected during prolonged action of artificial gastric juice, which also does not dissolve the mass at the pole of the cell. The latter gives a distinct reaction for "masked" iron. This result indicates that the substance forming the mass is not related to the substance, cyanophycin, forming the granules of the second type, and that, therefore, Borzi's supposition as to the composition of the mass is incorrect. The heterocyst is, therefore, a degenerated cell. It may also possibly represent more than this. Its formation next to the spore in *Cylindrospermum majus* and other forms, as well as its development beside the cell out of which arises the lateral trichome branches in *Tolypothrix* would appear to suggest that the heterocyst may be the result of some rudimentary sexual process.

VII.—CELL DIVISION.

Ordinarily the first sign of cell division in the Cyanophyceæ is the growth inward from the lateral wall of a septum which appears thus to separate, not only the cytoplasm as a whole, but also the central body, into two equal parts. It is, however, only in such genera as *Tolypothrix* and *Scytonema*, in which the cells are, in comparison to their length, very long, that one is able to ascertain what are the earliest phenomena of cell division. In preparations of trichomes from quickly growing cultures one may find a cell in which the deeply stained central body has a constriction which gives it a slight hour-glass appearance. In some other cells, perhaps in the same trichome, the constriction may be greater and at the same time the ingrowth from the lateral membrane to form the transverse septum may be found. The constriction of the central body, before the appearance of a trace of a septum, must be held to indicate that the central body initiates division.

The mode of the formation of the transverse septa explains the central perforation or opening in the septa which Borzi saw. In old septa I have never observed these passages or perforations. They are really due to incomplete formation of the septa.

In the division the granules are apportioned to the daughter cells according to their distribution. There is no rearrangement, no grouping

of them, nor any change so far as can be determined in their chemical reaction. It may happen, as it often does in *Microcoleus terrestris*, that while the granules of the second type are equally divided between the daughter cells, one of the latter may receive all the granules of the first type. When, however, these granules are very large in proportion to the cell which contains them, such granules are divided when the cell divides. Instances of this were seen in some preparations of *Oscillaria natans* found in a rapidly growing condition and treated with artificial gastric juice for from eight to ten hours. In these there were very large granules, or rather spherules, of the first type, which occupied a large portion of the cell space and which stained deeply in hæmatoxylin. In the undividing cell they were spherical, or nearly so, but in those dividing as well as in those which had divided, they exhibited a constriction in the plane of the transverse septum formed but not completed, while in other cases where the septum was fully formed, each daughter cell had its half of the original spherule or granule. (Fig. 19). It is obvious that in such cases the division of the spherules was not physiological but mechanical and that it followed the division of the cytoplasm.

Such instances of division of the granules are rare and would not, it must be believed, occur in the large-celled *Oscillariæ* if the granules or spherules were not correspondingly large, a condition which does not occur. It is, however, interesting to note that in one form of the *Cyanophyceæ*, at least, the substance in the cells which represents chromatin in these organisms, when present in abundance is divided between the daughter cells in a mechanical way and after the daughter cells are formed.

SUMMARY.

1. In the living cells of Cyanophyceæ, with the exception of those in which the transverse diameter of the trichome is very minute, two zones can be readily made out: one central, uncoloured, the other peripheral, holding the pigment. The cytoplasm forming the central zone, or body, is denser than that present in the peripheral layer.

2. The pigment is dissolved in a fluid which occupies vesicles in the cytoplasm of the peripheral layer. There is no evidence of the presence of a special chromatophore.

3. The central body is finely vesiculated and, except in its periphery, almost free from granules. In it obtains a small quantity of a chromatin-like substance which resists the action of artificial gastric juice and contains organic phosphorus and "masked" iron. This substance is uniformly diffused throughout the cytoplasm of the central body.

4. The cytoplasm of the peripheral layer is, compared to that of the central body, always somewhat coarsely vesiculated. It gives a very faint reaction for "masked" iron and a feeble reaction for organic phosphorus. In the part immediately adjacent to the central body the reactions for both are slightly deeper than that demonstrated in the outermost part of the peripheral layer.

5. The granules present are usually of two types, one of which is formed of a substance staining with hæmatoxylin and containing "masked" iron and organic phosphorus and, therefore, resembling chromatin. They are, on prolonged digestion with artificial gastric juice, dissolved. Granules of this, the first type, when large, are found to be hollow spherules. These granules are usually found in the peripheral portions of the central body, but they are not confined to that part, for they may rarely be observed in the central parts of the central body and also in the inner zone of the peripheral layer in some forms.

6. The granules of the second type are to be found in the peripheral layer and chiefly adjacent to the cell membrane. Rarely are they hollow spherules. They are constituted of a substance which stains deeply with picrocarmine and is free from organic phosphorus and "masked" iron. This substance dissolves very quickly in weak acids. As it gives a reaction for sulphur when treated with plumbic acetate in alkaline solution, it is probably a proteid.

7. In one form, *Cylindrospermum majus*, only one kind of granules is

present and these are found in the peripheral layer. The substance which forms these stains deeply with picrocarmine and with difficulty with hæmatoxylin. It appears to contain "masked" iron.

8. The heterocyst is a degenerated cell in which all distinction between the central and peripheral parts is lost. The chromatin-like substance of the central body diffuses throughout the cytoplasm when the heterocyst is forming. When fully developed the cytoplasm gives a feeble reaction for iron. A small mass at one or either pole of the cell gives a distinct reaction for "masked" iron and stains deeply with picrocarmine. Further, as it does not dissolve in acids, it is not related to the substance which forms the granules of the second type.

9. There is no nucleus, nor any structure which resembles a nucleus, in the Cyanophyceæ.

10. Division of the cell is direct, the central body first showing the effects of this process. When a large spherule of chromatin-like substance is present it may pass into a daughter cell, or it may be mechanically divided between the two daughter cells.

BEGGIATOEA.

LITERATURE.

The structure of *Beggiatoea* is to a great extent recognized as affecting the question of the structure of Bacteria in general, and consequently in the literature on the subject one finds frequent reference to the structure of various species of *Beggiatoea*. Some of these references are, however, of a very brief character and deal only with the external form. The more important observations bearing on the internal structure are those of Bütschli,¹ Mitrophanow² and Fischer.³

According to Bütschli in *Beggiatoea*, as in other sulphur Bacteria and in the Cyanophyceæ, the cytoplasm of every cell has a central body and a peripheral zone. These two structures are quite distinct in properly made preparations. In the central body are frequently large lacunar spaces which in the living cell are occupied by the sulphur granules. The central body also contains minute granules which in position and staining properties correspond to the "red" granules of the Cyanophyceæ. In some preparations of *B. alba* the peripheral zone was very narrow, so much so that the central body appeared to come almost in contact with the membrane of the cell. In *B. mirabilis* the central body is extraordinarily large and in it is a large vacuole in the interior of which, in the living cell, are small pale corpuscles in molecular movement. On the surface of the central body is a single layer of vesicles (Waben). Minute "red" granules are also found in the central body.

Bütschli holds that the central body, thus described, is the analogue of the nucleus of more highly specialized cells and that consequently the peripheral zone of cytoplasm corresponds to the ordinary cell protoplasm of higher organisms.

Mitrophanow describes as nuclei of the Sulphur Bacteria structures of the most diverse form and character. In some cases, in *Chromatium* and in *Rhabdochromatium* for example, it is an irregular, centrally placed mass, elongated parallel to the long axis of the cell. In other cases it

¹ "Ueber den Bau der Bacterien und verwandter Organismen," Leipzig, 1890. Also, "Weitere Ausführungen über den Bau der Cyanophyceen und Bacterien," Leipzig, 1896.

² "Études sur l'organisation des Bactéries," Intern. Monatschr. für Anat. und Physiol., Vol. X, p. 475, 1893.

³ Untersuchungen über den Bau der Cyanophyceen und Bakterien," Jena, 1897.

contains a collection of granules of chromatin which stain differently from the substance in which they are held, and more deeply. Not unfrequently there may be several masses sometimes completely separate from each other, in other cases connected by narrow strands of the same substance. In some cells also the granules which Mitrophanow terms "nucleolar" are uniformly distributed throughout the cytoplasm of the cell. In such no nucleus is visible. In *Ophidomonas jenensis* the central elongated mass reminds one of the central body of Bütschli, the peripheral cytoplasm corresponding to the peripheral layer of that author. This mass may appear divided into a number of smaller vesiculated irregular clumps of substance. In *Beggiatoa* the masses are always spherical, and, to judge from Mitrophanow's figures, homogeneous in composition. Several of these may be present in the cell, though as a rule there are not more than two large ones.

Mitrophanow's terminology is very obscure. He applies the term nucleus to the large elongated, centrally placed mass and the term nucleoli to the granules which it may contain, or to those which may be distributed throughout the cytoplasm. In the case of *Beggiatoa* the granules found are loosely described by him as nucleoli. He has advanced nothing, except, perhaps, facts in regard to staining capacity, which justify the application of the term nucleus to these structures.

In neither *Chromatium* nor *Beggiatoa* could Fischer find a differentiation of the cytoplasm into central body and peripheral layer, such as Bütschli describes. In *Chromatium* there may at times be a condensation of the cytoplasm in the centre of the cell, brought about by the arrangement and disposition of the sulphur droplets which it contains. There may be in each cell a number of spherical grains of a deeply stainable substance which Fischer hesitates to regard as chromatin, for after the use of some chromatin-fixing reagents the granules formed of it are not to be seen. In *Beggiatoa* Delafield's hæmatoxylin brings out the presence in each cell of "red" granules such as Bütschli describes. In subsequent treatment of these preparations with safranin the central portions of the cells stain a little more deeply than their periphery, but this is due to condensation of the cytoplasm through the disposition of the sulphur granules. In cells free from sulphur this result is not obtained. There is no nucleus and the granules cannot definitely be regarded as formed of chromatin.

The writer¹ pointed out that the "masked" iron compound is dis-

¹ "On the Distribution of Assimilated Iron Compounds, other than Hæmoglobin and Hæmatins, in Animal and Vegetable Cells," Quart. Journ. Micro. Sci., Vol. XXXVIII, p. 258.

tributed uniformly throughout the cytoplasm and that this distribution corresponds with the diffuse stain given by hæmatoxylin. In the "comma" forms granules which stained with hæmatoxylin gave a reaction for "masked" iron.

MATERIAL AND METHODS OF STUDY.

The forms used were *Beggiatoa media*, *B. alba* and *B. mirabilis*. The cultures of the two former in water containing sulphuretted hydrogen in solution were always kept in the actively growing condition. These cultures could in twenty-four hours be got to yield myriads of the spirillum-like elements, the "comma" forms, and the "cocci," which, according to Zopf¹ are different stages in the development of *Beggiatoa*.²

One method of fixation was to place a drop of the culture on a cover glass, allow the water to evaporate, and then to float the cover, preparation surface downwards, on a saturated solution of corrosive sublimate, where it was left for a couple of hours, after which it was passed successively through alcohols of fifty, seventy and ninety per cent. strengths. Cover glass preparations were made with ninety-five per cent. alcohol, without the employment of any other reagent. Picric acid in saturated solution was also employed on cover preparations as well as on quantities of the material in various stages. Material in bulk was hardened in ninety-five per cent. alcohol alone.

In staining, methylene-blue, safranin, eosin and Ehrlich's and Heidenhain's hæmatoxylin were employed. The first three dyes, each used alone, give results of no value, but they may individually be employed with one of the hæmatoxylin and thereby a more marked demonstration of the vesicular structure of the various forms may be obtained. If, however, the iron-alum hæmatoxylin method alone is carefully employed it will give preparations which in distinctness leave nothing to be desired.

The material from *B. mirabilis* was hardened, part in absolute alcohol and part in picric acid in saturated aqueous solution.³

¹ "Zur Morphologie der Spaltpflanzen," Leipzig, 1882. Also "Die Spaltpilze," Breslau, 1883.

² These forms are, according to Winogradsky ("Beiträge zur Morphologie und Physiologie der Bacterien," Leipzig, 1888), not genetically related to *Beggiatoa*. He was unable to find a transformation of the *Beggiatoa* threads into the "cocci" forms, an experience which befell Engler ("Ueber die Pilz-Vegetation des weissen oder todtten Grundes in der Kieler Bucht," Vierter Bericht d. Commission zur Wiss. Untersuchung der deutschen Meere in Kiel, p. 185, Berlin, 1884).

³ For this material I am indebted through Dr. E. C. Jeffrey to Mr. Billings.

GENERAL CELL STRUCTURE.

In fresh actively growing specimens of *B. alba* the cytoplasm of the filaments is crowded with minute sulphur droplets, and it is difficult to determine the presence of any other structures. One can, with very high powers and good illumination of the microscopic field, see the transverse septa marking the thread off into cells, and at the same time find the cytoplasm next these septa free from granules.

In the fixed preparations which have been passed through alcohol and stained the result is different. In Fig. 60 are represented three cells of a thread of *B. alba*. The sulphur has been removed by the alcohol and the places occupied by the sulphur are shown as clean vacuoles. The protoplasm near the transverse septa appears denser than elsewhere, although because of the aggregation of the vacuoles around the centre sometimes the cytoplasm at the latter point gives an appearance of condensation. The stain taken by the cytoplasm is uniform throughout the cell, but fine granules may not unfrequently be observed. I am, however, unable to corroborate Mitrophanow regarding such large granules as he illustrates. When the threads become less rich in sulphur and therefore, ill nourished, large granules may be found, not quite so often indeed, as he observed, but still much more frequently than in the preparations from actively growing cultures. I am inclined to believe that Mitrophanow's preparations were made from ill-nourished cultures, and an examination of his illustrations (Figs. 28 and 30) convinces one of this, for in them is an utter absence of such vesiculation as would be present, had the cells, when prepared, contained sulphur droplets.

There is no evidence whatever of the presence of any nuclear structure. The cells of well-nourished threads in no case show a differentiation of their cytoplasm into central and peripheral parts according to the views of Bütschli. It is rare even to get, as Fischer did with hæmatoxylin and safranin, a slightly deeper stain in the central part, and when this does appear it is due to the fact that the central part of the cell is seen through a greater quantity of cytoplasm than is the periphery of the cell.

In *B. mirabilis* hardened in alcohol the cell frequently contains a slightly denser portion of cytoplasm placed adjacent to one of the transverse septa, but an examination of this mass shows that it is really a shrunken portion of the cytoplasm which filled the whole cell. When

the preparations are made with picric acid the frequency of these shrunken masses is greatly reduced. The cytoplasm then usually appears vesiculated throughout and it stains uniformly with hæmatoxylin, showing an absence of granules.

When the threads of *B. alba* and *B. mirabilis*, after being hardened in alcohol, were treated with the nitric-molybdate reagent for two to three hours and then acted upon with a solution of phenylhydrazin hydrochloride of two per cent. strength, in order to demonstrate the distribution of organic phosphorus, the latter was found to be uniformly diffused throughout the cell. In *B. mirabilis* the condensed portions observed in some cells, as already described, gave a marked indication of the presence of "masked" phosphorus, but it appeared so because in the shrunken condition to which these masses are due more cytoplasm is gathered into a smaller volume than in cells with unshrunken cytoplasm. Fig. 61 illustrates this. In *a* and *b* are observed two shrunken masses of cytoplasm, in these are aggregations simulating granules, while in *c* the cytoplasm shows the organic phosphorus uniformly distributed throughout the cell. In *B. alba* the phosphorus is distributed uniformly with the cytoplasm and the method did not reveal the presence of granules.

The reaction for iron derived from the "masked" condition, is in *B. alba* and *B. mirabilis* found to be uniform with the distribution of the cytoplasm. When sulphuric acid alcohol is used to set the organic iron free in the threads and the preparation is washed free from acid with absolute alcohol and stained with a pure aqueous solution (one per cent.) of hæmatoxylin, the result is decided enough to determine definitely that there are no specialized chromatin-holding structures like nuclei or like Bütschli's central body. Granules sometimes found distributed in the peripheral portion of the cytoplasm give the reaction.

The distribution of the "masked" iron being then like that of the organic phosphorus, it follows that the substance containing these elements, the analogue of the chromatin of more highly specialized cells, is contained, not in any nucleus however rudimentary, but diffused in the cytoplasm, and sometimes, also, localized in granules.

Somewhat different are the results of observations on the spirillum-like form, and on the "cocci," and comma-shaped organisms. Here, as little as in the thread or "leptothrix" forms, is there any evidence of the existence of a nucleus, rudimentary or otherwise. In these, the vesicles occupied by the sulphur droplets, are all crowded about the centre, or about the central axis, leaving the peripheral layer as a thin, homogeneous structure applied to the membrane. In the "spirillum" form the

vesicles are separated from each other by a thin film of cytoplasm, and here and there, and especially at the nodal points in these films, one observes, in hæmatoxylin preparations, granules which are more deeply stained than the rest of the cytoplasm. Sometimes these granules are more or less drawn out in the films between the vesicles, and then the central cytoplasm may take on the character of the central body of Bütschli. Of the actual existence of such a central body there is not the slightest evidence.

In the "comma" forms the granules are very much fewer, and frequently smaller. In the "cocci" they are rarely visible, and minute lightly stainable granules make their appearance at the periphery of the vesicles (Fig. 59, *a*, *b*, and *c*).

In the "spirillum," as well as in the "comma" form, the compounds of "masked" iron and organic phosphorus are, apart from the granules, faintly diffused throughout the cytoplasm, sometimes apparently less abundant in the central portions of the cells, and definitely indicated in the peripheral layer. The granules give a slightly more marked reaction for "masked" iron as well as for organic phosphorus, and this fact, combined with their capacity for taking up hæmatoxylin, would seem to indicate that they are formed of a compound analogous to chromatin.

The difference between the structure of the threads, and that of the "spirilla" and the "comma" forms in regard to granules, may be due to some inherent difference in the process of nutrition in the two different types, but it may also be explained on the view of Winogradsky that these types are not genetically connected, that they belong to different species of Sulphur Bacteria.

Whether this is correct or not does not affect the question that, in all these types of structure, there is nothing to simulate a nucleus, even of the most rudimentary description. It can scarcely be contended that the granules, the affinity of whose substance to chromatin is shown by their containing, in a small degree, "masked" iron and organic phosphorus, are morphologically of the value of nuclei, or even of nucleoli, as Mitrophanow appears to claim.

SUMMARY.

1. In *Beggiatoa alba* and *B. mirabilis* there is no differentiation of the cytoplasm, as Bütschli finds, into a central body and a peripheral layer. The centrally placed portion of the cytoplasm contains, in the well-nourished fresh cell, a number of sulphur droplets, and frequently the cytoplasm between the droplets may be more condensed than at the periphery of the cell, but usually both portions stain equally deeply.

2. The compounds of "masked" iron and organic phosphorus are uniformly and equally diffused throughout the cytoplasm in the threads of *B. alba* and in *B. mirabilis*, and the organic phosphorus is found uniformly distributed in those cells which contain unshrunk cytoplasm. When granules which stain with hæmatoxylin occur, they are found to contain "masked" iron and organic phosphorus.

3. In the "spirilla," in the "comma" forms and in the "cocci," the cytoplasm shows characters like the cytoplasm of the threads, but there are, in addition, granules which give slight reactions for "masked" iron and organic phosphorus, and which, therefore, are constituted of a substance analogous to chromatin.

4. There is, in all these forms, no specialized chromatin-holding structure in the shape of a nucleus of any kind.

THE YEAST CELL.

I.—LITERATURE.

The structure of the Yeast Cell, and more especially the question whether it has a nucleus or not, has been the subject of investigation by a large number of observers since 1844, when Nägeli¹ affirmed the existence of a nucleus in this organism. Chief amongst these were Schleiden² (1849), Brücke³ (1861), Schmitz⁴ (1879), Strasburger⁵ (1884, 1887), Zalewski⁶ (1885), Krasser⁷ (1885 and 1893), Hansen⁸ (1886), Zacharias⁹ (1887), Zimmermann¹⁰ (1887), Raum¹¹ (1891), Moeller¹² (1892 and 1893), Hieronymus¹³ (1893), Janssens¹⁴ (1893), Dangeard¹⁵ (1893 and 1894), Macallum¹⁶ (1895 and 1898), Buscalioni¹⁷ (1896), Wager¹⁸ (1897

¹ Zeit. für Wiss. Botanik, Vol. I, p. 45, 1844.

² "Grundsätze der Wiss. Botanik," 1849, p. 207.

³ "Die Elementar-organismen." Sitzungsber. der K. Akad. d. Wiss. zu Wien, Math.-Nat. Classe, 1861, Vol. XLIV, Abth. 2.

⁴ "Untersuchungen über den Zellkern der Thallophyten," Sitzungsber. der Niederrhein. Gesell. für Natur- und Heilkunde zu Bonn, Sitzung am 4 Aug., 1879.

⁵ "Das Botanische Practicum," p. 339, 1887. Also edition of 1884.

⁶ "On Spore Formation in Yeast Cells," Transactions of the Scientific Academy of Cracow. (Polish). Vol. XIII, 1885. Abstract in Bot. Centralbl., Vol. XXV, p. 1, 1886.

⁷ "Ueber das angebliche Vorkommen eines Zellkerns in den Hefezellen." Oestereich. Bot. Zeits., 1885; also: "Ueber den Zellkern der Hefe;" *ibid.*, 1893, p. 14.

⁸ "Recherches sur la Physiologie et la Morphologie des Ferments Alcooliques." Meddeler fra Carlsberg Laboratoriet, Vol. II, p. 152, 1886.

⁹ "Beiträge zur Kenntniss des Zellkerns und der Sexualzellen." Botanische Zeitung, 1887, Nos. 18-24.

¹⁰ "Die Morphologie und Physiologie der Pflanzenzelle." Breslau, 1887, p. 25.

¹¹ "Zur Morphologie und Physiologie der Sprosspilze," Zeitschr. für Hygiene, Vol. X, p. 1, 1891.

¹² "Ueber den Zellkern und die Sporen der Hefe," Centralbl. für Bakt. und Parasitenkunde, Vol. XII, p. 337, 1892; also: "Weitere Mittheilungen über den Zellern und die Sprosse der Hefe;" *ibid.*, Vol. XIV, p. 358, 1893.

¹³ "Ueber die Organization der Hefezellen." Ber. d. deutsch. Bot. Gesell., Vol. XI, p. 176, 1893.

¹⁴ "Beiträge zu der Frage über Kern der Hefezelle," Centralbl. für Bakt. und Parasitenkunde, Vol. XIII, p. 639, 1893.

¹⁵ "Sur la structure histologique des levures et leur développement." Comptes Rendus, Acad. d. Sciences, Vol. CXVII, p. 68, 1893; also: "La structure des levures et leur développement," Le Botaniste, 1894, p. 282.

¹⁶ "On the Distribution of Assimilated Iron Compounds, other than the Hæmoglobin and Hæmatius, in Animal and Vegetable Cells," Quart. Journ. Micro. Sci., Vol. XXXVIII, p. 243, 1895; also: "On the Detection and Localization of Phosphorus in Animal and Vegetable Tissues," Proc. Roy. Soc., Vol. 63, p. 467, 1898.

¹⁷ "Il Saccharomyces guttulus Rob." Malpighia, Vol. X, 1896.

¹⁸ "The Nucleus of the Yeast Plant," Report British Association, Toronto Meeting, 1897, p. 860; also: *ibid.*, Bristol Meeting, 1898, p. 1,069, and Annals of Botany, Vol. XII, p. 499, 1898.

and 1898), Janssens and Leblanc¹ (1898), and Bouin² (1898), a fairly full account of whose observations, as of those of others, is given by Wager (1898) to which the reader may be referred. Many of the observations, especially those of more than ten years ago were made with imperfect means and methods, and although there is amongst the older observers almost an unanimous agreement on some points, as for example, the presence of a nucleus, it must be now recognized that with the employment of the simple methods alone used by them, no observer can maintain with the same degree of certainty that he can see the structures which they found, or can admit that what they found is what they claimed it to be. I propose here, therefore, to limit any discussions of the observations to those made within the last seven years, and particularly to those of Moeller, Buscalioni, Wager, Janssens and Leblanc and Bouin, for these observers not only gave thorough attention to the structure of the yeast cell, but also used very much improved methods of fixation and staining. I may add in justification of my not giving an account of the earlier observations that in 1895 I discussed these at some length.³

Moeller in his first paper claimed that the yeast cell contains a nucleus which is homogeneous and without a membrane. This nucleus changes its shape readily, and, therefore, its position in the cell varies. Owing to this property it may assume a thread-like form when budding occurs, a portion of which is thus enabled to pass into the protoplasm of the bud through the narrow connecting tube. The part which projects into the bud breaks off and separates with the bud, assuming finally the rounded form of the mother nucleus. In the spores, however, Moeller could not find any evidence of the presence of a nucleus, but in his later communication he states that he found the nucleus in the spore element, and he describes its character. The nucleus of the cell, at the beginning of spore formation, enlarges and becomes elongated and constricted at the middle. The constriction deepens, the ends separate to the opposite poles of the cell, and the fine thread joining the two parts breaks, two daughter nuclei being thus formed. A second and similar division follows. The division is a direct one.

Janssens found in *S. cerevisiae* and in *S. Ludwigii* a nucleus provided with a homogeneous nucleolus and membrane, the diameter of the nucleolus being one-third that of the nucleus. The nucleus divides

¹ Recherches cytologiques sur la cellule de levure." La Cellule, Vol. XIV, p. 203, 1898.

² "Contribution à l'étude du noyau des levures," Arch. d'Anat. Microscopique, Vol. I, p. 435, 1898.

³ Quart. Journ. Micro. Sci., Vol. XXXVIII, p. 243.

in the mitotic fashion, and Janssens claims to have observed the equatorial plate and dyaster stages. In spore formation the nuclear membrane disappears, and the plane of the second division is at right angles to that of the first, the two spindles also being at right angles to each other. Each spore is provided with a nucleus.

Later Janssens, in conjunction with Leblanc, published a fuller and somewhat different account of the structure of the cell. They found in *S. cerevisiae* and *S. Ludwigi* a nucleus with a distinct nuclear membrane, caryoplasm, and a nucleolus constituted of nuclein. The caryoplasm is formed of a fine network of fibrils intimately connected with each other and applied to the nucleolus. When, however, the cells are put in a fresh culture medium, the nucleus becomes vacuolized, but the nucleolus maintains its shape and central position, and the protoplasm remains homogeneous. The vacuolated condition ceases at about the thirteenth hour. In a longer stay in the medium the protoplasm becomes granular. Ordinarily the cytoplasm is formed of a typical reticular structure, the meshes of which contain granules. Both the contents of the meshes as well as the reticulum and its nodal points, in some cases, manifest a strong affinity for colouring matters, and it is evident that this is due to a nucleo-albuminous substance dissolved in this structure. When the cells are grown on plaster blocks the granules may become very large and refracting, but when sporulation begins these granules disappear, presumably contributing a portion of the material which constitutes the spores. Glycogen in the cell ordinarily is dissolved in the enchylema, but when it is very abundant it localizes itself in vacuoles which may fill the cell. When the cell buds, the nucleolus elongates and divides, but the two parts remain united by a strand of substance apparently like fibrils, which may slightly resemble a spindle. The two nucleoli pass toward that part of the cell which is giving rise to the bud. The nuclear membrane and the caryoplasm disappear, leaving the nucleoli nude. A structure which resembles, to a certain extent, a cell plate, makes its appearance in the cell between the two nucleoli. The process up to this point is a rudimentary form of kinesis. One of two nucleoli slips into the bud, and now, if not before, the nuclear membrane re-forms about both. The bud thus constituted is, in all respects, like the mother cell.

In the formation of the spores, the authors find that early in the spore mother cell the nucleolus divides, as already described, but the nuclear membrane does not disappear. This is not accompanied by any division of the cell. After some hours, however, only one nucleus with a large nucleolus is observed. The authors believe that the two nucleoli

originally present have fused or conjugated, that is, fecundation has taken place. This is followed by division of the large nucleolus, the elongation taking place in the long axis of cell. In each of the two nucleoli thus formed a second division occurs, immediately following the first, the elongation of the two daughter nucleoli being at right angles to each other. When the four nucleoli are fully formed the membrane develops about each, and around each of the nuclei thus constituted the protoplasm collects, a membrane finally forming about each mass, which becomes a spore.

According to Buscalioni, whose observations were made on *S. guttulatus*, the yeast cell contains a nucleus which ordinarily is homogeneous, and its division is by constriction. This obtains when budding occurs, the two daughter nuclei remaining connected by a thin fibril until one of them enters the bud. This is simple fragmentation. In the formation of spores, however, the nucleus undergoes a slightly different species of division which may be looked upon as a rudimentary kind of kinesis.

Bouin found a sharply defined nucleus which, during fermentation sends prolongations into the cytoplasm. The latter are less sharply defined the further they proceed from the centre of the nucleus. The nucleus ordinarily may be granular, or may contain in its interior irregular, deeply stainable masses. In some cells a nucleus would appear to be absent. In these an intense stain may serve to show a nucleus poor in chromatin; but cells which do not appear to have a nucleus have lost it by its transference, without division, into the bud. The nucleus may, under certain conditions, divide and re-divide, while the cell may remain undivided, and Bouin holds that this multiple division of the nucleus accounts for the number of chromatin granules found in some yeast cells. The granules are, in this case, nuclei, and the cell containing them, multinucleate. The division of the nucleus ordinarily is amitotic, that is, there is elongation of the nucleus with constriction, the thread uniting the two ends becoming more and more delicate till rupture occurs. One of the two daughter nuclei thus formed passes through the canal between mother cell and bud, and into the latter, where it becomes spherical. No striation of the cytoplasm between the two daughter nuclei was observed, nor was any evidence of an equatorial plate and of chromosomes found. In the formation of spores the nucleus divides into two chromatin masses, which separate, then become rounded, and constitute the nuclei of the spores. This mode of division is intermediary between mitosis and amitosis.

Wager, from his first observations, claimed that in *S. cerevisiae* a spherical homogeneous nucleus is to be found placed between the cell wall and the vacuole, which, after digestion in pepsin-glycerine solution, reveals a granular structure. From this he concludes that it consists of deeply stainable granules imbedded in a less stainable matrix. The granules are probably formed of chromatin. In budding, the nucleus divides directly, and this occurs in the narrow passage between the mother cell and the bud. When division is to take place, the nucleus places itself opposite the opening and proceeds to make its way into the bud, until about half of it has passed through, when it divides completely, the products constituting nuclei for the mother cell and the bud. In the cell about to sporulate the large vacuoles disappear, the protoplasm is beset with small ones, and the homogeneous nucleus is centrally placed. In it, granules, however, soon make their appearance, which accumulate in the centre, and look like a nucleolus. In division, the outline of the nucleus becomes irregular, and the granules arrange themselves in the form of a short rod, surrounded by other portions of the nucleus, which stain differently, and appear to form a structure like a spindle. The granules form two groups, each of which constitutes a nucleus, and each of the two nuclei divides in the same way, forming thus the nuclei of the spores. Around each of these nuclei protoplasm accumulates and a membrane forms. This form of nuclear division Wager regards as a simple form of karyokinesis. In *S. Ludwigii*, he found a nucleus with membrane, and a nuclear network and nucleolus the latter containing all the chromatin. In division, the nucleolus increases in size, and divides, each part becoming a nucleus.

In the paper detailing his later observations, Wager gives a fuller, and, in some respects, a considerably different account of the yeast cell. In the fresh, actively growing organism the cell contents are clear and homogeneous, with sometimes one or more bright refracting granules. In this condition a vacuole or vacuoles can be seen, and in each occurs at least one refracting particle which is in a state of movement. The vacuoles disappear in a later stage of fermentation, and the protoplasm then appears homogeneous and clear; but, when the culture medium becomes exhausted, the contents become granular and possess fat globules, the protoplasm shrinks from the cell wall, and the cell presents an appearance of disintegration. In compressed yeast, on the other hand, the cells are rich in refracting granules, which are sometimes uniformly distributed through the protoplasm, sometimes located around the vacuoles, or grouped together at one side of the cell.

In regard to the nuclear apparatus, Wager distinguishes two struc-

tures: one of which he calls the nuclear body, while he terms the other the nuclear vacuole. The nuclear body, which is the nucleus of Moeller, and of his own earlier observations, is homogeneous, but surrounded more or less completely by granules, and which, with low powers of magnification, give it a granular appearance. It is, in actively growing cells, usually in close contact with the cell wall, but it may, in a few cells, be more centrally placed. In relation frequently to this nuclear body there are granules, first described by Hieronymus, some of them of an oily nature, others of a proteid character, sometimes grouped about the nuclear body, sometimes in its immediate neighborhood or distributed throughout the cell. At times these form a coiled thread. The nuclear vacuole, which is in contact with the nuclear body in growing cells, contains chromatin, sometimes in the form of granules, sometimes in the form of a network, sometimes as an irregularly shaped mass attached to the wall of the vacuole by fine threads. In some cells all the chromatin substance appears to reside in the vacuole; in others it is diffused through the protoplasm, and in some cells again it appears in the nuclear body. The vacuole Wager regards as the nucleus of Janssens and Leblanc, and the nucleolus of these observers constitutes his nuclear body. The nuclear vacuole may persist but for a short time. After fermentation has proceeded for some hours it disappears, and its place is occupied by a granular network in contact with the nuclear body. The vacuoles seem to arise by fusion of minute vacuoles which develop in connection with what appears to be chromatin granules.

In regard to division, Wager found that the nucleolus (the nuclear body) is separated from the bud by the vacuole, which, as the bud develops, begins to pass into it. At the same time, the nucleolus makes its way to the base of the opening, and there, or in the neck, at once begins to elongate and constrict for division. The vacuole at this time divides, but not completely or equally, the smaller portion being found in the daughter cell, both parts remaining connected by a granular thread. The divisions of the nuclear body are nearly or quite equal, and one of them makes its way into the daughter cell. When the nucleolus is in the neck, the constriction takes place with the ends in the mother and daughter cells. When there is no vacuole, the granular network in contact with the nuclear body undergoes division into two more or less equal portions, either in the mother cell or in the neck of the bud. The granules which the young bud thus receives seem to develop, in some way, the vacuoles which form the single large nuclear vacuole.

In sporulation the nuclear vacuole disappears, or its place is taken by

two or more smaller ones which in turn are replaced by many others and, as a consequence, the protoplasm acquires the foam structure of Bütschli. The nuclear body moves towards the centre, the protoplasm condenses about it, and on the periphery of this condensed zone deeply stainable granules collect. The nuclear body now appears to take up from the surrounding cytoplasm all the stainable material, and to deposit it in its centre as a granular mass. The division of the nuclear body occurs as Wager describes it in his earlier paper.

In regard to the nature of the nuclear apparatus, Wager regards it as a simple form of a nucleus, although he admits the possibility of it being either a primitive structure representing an early stage in the organogeny of the nucleus, or a degenerated form of nucleus. The division of the nuclear body, he thinks, may be regarded as a case of direct division, but, in his opinion, it may also be a very simple case of karyokinesis.

In my own studies on the distribution of assimilated compounds of iron, I pointed out that, in *S. cerevisiae* and *S. Ludwigii*, chromatin is to be found distributed throughout the cytoplasm of the cells and, sometimes, also in the latter in the form of granules; but, in *S. Ludwigii*, it may be found chiefly at the periphery of each large vesicle, when only a few large vesicles are present in the cell. In this form also there is a substance constituting corpuscles of a nucleolar character, the nuclei of Moeller, which stains with eosin, and gives a marked reaction for iron, but differs from chromatin in remaining unstained after treatment with hæmatoxylin. My conclusion was that there is no nucleus, although such an organ may occur in other stages of this organism. In a later contribution embodying the results of observations made to determine the distribution of organic phosphorus in animal and vegetable cells, I pointed out that in the yeast cell the phosphorus-holding substance, or nucleo-proteid, although sometimes in the form of granules or spherules which have been taken for nuclei, is frequently dissolved in the cytoplasm.

It will be seen from this abstract of the more recent literature on the yeast cell, that there are some discrepancies in the views of various investigators of the subject. An agreement is indeed found as regards the division of the nuclear body or nucleolus, but not as to the manner of this division. The most radical difference, perhaps, exists between Wager, on the one hand, and Janssens and Leblanc on the other, as to what constitutes the nucleus, and as to its structure apart from the nuclear body or nucleolus.

II.—METHOD OF STUDY, AND SPECIES STUDIED.

The fixing reagents which I used were corrosive sublimate and picric acid, each in saturated aqueous solutions, the chrom-osmio-acetic mixture of Flemming, and a one per cent. solution of potassic iodide saturated with iodine. Undoubtedly for yeast cells the best of these, in my experience, are corrosive sublimate and Flemming's fluid. They produce less alteration in structure than any other, and they do not, when properly used, alter the staining power of any part of the yeast cell. The most satisfactory method of employing them was to allow them to act in bulk on a large number of yeast cells, separated from the actively growing cultures by centrifuging the latter. After the reagents had acted sufficiently long the fluid was decanted, distilled water was poured upon the cells, which were immediately subjected to centrifuge action and thus separated, when they were treated with alcohols of gradually increasing strengths. With these reagents also I obtained reliable cover-glass preparations, in which drying of the cells was not a factor, by spreading a film of the yeast culture on the cover-glass, and then, with this face downward, placing it floating on a quantity of the solution, to remain there for from one to twenty-four hours. The fluid, at the moment of touching, removed very many of the elements, but enough were left adherent to make a good preparation. Afterwards the cover-glass so treated was placed in alcohol of 30, 50, 70, and 90 per cent. strengths successively.

The cover-glass method of preparation could not be employed with the other reagents except to some extent in the case of iodine, as these remove from the cover all the cells. The only safe way of fixing with these solutions was to allow them to act on the yeast cells in the test tube. They were separated completely from the fluid, after the required time for complete fixation, by centrifugalizing the fluid. The hardening in these cases was completed by the use of alcohols of 30, 50, 70, and 90 per cent. strengths successively. The cells were completely separated in these cases by gravity. In the case of the iodine solution the method employed by some of allowing a film of yeast cells to dry on a cover-glass, and then placing it in the reagent, has no advantage over the one described, and I am not sure that it is free from objection. It is difficult to believe that yeast cells can be unaffected when the fluid about them is completely removed by evaporation. I have, therefore, avoided this method of preparation, as well as that in which heat alone is used for the purpose of fixation.

With regard to iodine solutions more particularly, it is necessary to use a word of caution. The prolonged action which is required with this reagent affects the proteids of the cell, and must change consequently the reactions of the cellular structures to staining solutions. I have found that it alters and changes the staining capacity of the cell in *Spirogyra*, as well as of various animal tissues, and it can scarcely be admitted that it has no effect on the cytoplasm of the yeast cell. I find that it greatly diminishes the affinity of the chromatin distributed throughout the cell for hæmatoxylin and other dyes which, in the case of corrosive sublimate preparations, select only the chromatin. The reagent does, indeed, assist in fixing the yeast cells in such a way as to single out for special demonstration a spherical, more or less homogeneous body, known to certain investigators as the nucleus, and to others as the nucleolus or nuclear body; but this property depends on the power of the iodine to change the chemical character of the cytoplasm in a greater degree than that of the nuclear body which is homogeneous and dense.

Amongst the staining reagents which were used were hæmatoxylin, safranin, eosin, and acetic-methyl green and methylene-blue. The solutions of hæmatoxylin gave the best results and, more particularly, Delafield's, Ehrlich's, and Meyer's (hæmalum). The solutions were made very dilute, so much so that it required always from sixteen to eighteen hours' application of the fluid to bring out the full stain. The iron-alum hæmatoxylin of Heidenhain was also employed, and it is of value in revealing the structure of the cytoplasm of the yeast cell, but it is of no value as a micro-chemical reagent, and it does not show any sharp distinction between chromatin-holding and chromatin-free cytoplasm. Eosin was used as a counter stain to hæmatoxylin. Acetic-methyl green was employed on the fresh cells, and methylene-blue on the cover-glass preparations.

The organic iron and phosphorus compounds were demonstrated in the manner described in the case of the Cyanophyceæ. The yeast cells were, for this purpose, always hardened in alcohol. To reveal the distribution of organic iron the cells were mounted on a slide under a cover-glass, in a mixture of glycerine and fresh ammonium hydrogen sulphide, and the preparation kept at a temperature of 60°C. for a week. To demonstrate the organic phosphorus, the cells were kept in a solution of ammonium molybdate in nitric acid for five hours, after which they were washed in distilled water for a few minutes, and then subjected to the action of a 2 per cent. solution of phenylhydrazin hydrochloride, which converted the molybdic portion of the phospho-molyb-

date into a greenish-blue compound. The cells, washed with distilled water and dehydrated, were mounted in balsam.

The species studied were *Saccharo myces cerevisia*, *S. Ludwigii*, and two found in cultures obtained from the throat in suspected cases of diphtheria. The specimens of *S. cerevisia* and *S. Ludwigii*, employed for the purposes of preparation, were in the actively growing condition in Pasteur solutions, from which a quantity of the cells, separated by centrifugalization, was taken hourly, starting with the commencement of growth, up to the twentieth hour, and treated as indicated above. Cultures of *S. Ludwigii*, in the sap of the iron-wood tree, *Ostrya virginica*, and of the maple, gave very valuable and instructive preparations. For the study of sporulation *S. cerevisia* was used, the sporulation having been brought about by cultivation in a 5 per cent. solution of sugar, as indicated by Wager.

III.—GENERAL CELL STRUCTURE.

In the fresh yeast cell at the beginning of fermentation, even with the highest powers of magnification, very little can be made out, except the occurrence of granules and vacuoles. These are grouped irregularly in the cell and their number and character may vary, although as a rule there is but one large vacuole. In some young, actively growing yeast-cells, that is in those which are observed two hours after the commencement of fermentation, there may be no vacuoles observable. In such, however, one or more granules may be found. This condition may be observed also in cultures of from sixteen to twenty hours.

Many of these granules appear to possess a fatty nature. When the yeast cells of this stage are hardened in Flemming's fluid for twenty-four hours the granules take a dark tinge, due apparently to the reduction of the osmic acid derived from the solution. They are not nearly as numerous in preparations hardened in alcohol as they appear to be in the fresh cell. At a later stage of fermentation the granules present are of a different composition. They do not react, or at most react but slightly, with the osmic acid of Flemming's fluid. They seem to be of a purely proteid character, for when the hardened cells are heated with a solution of potassic plumbate the granules acquire a light brown colour, this fact indicating the presence of organic sulphur. These granules were found to react also with freshly made Millon's reagent in from eight to ten hours without the application of heat.

It is of course possible that granules which give the reaction with osmic acid do not consist wholly of fat, for the reaction in all cases is not intense enough to suggest a purely fatty composition. The basis of apparently all the granules seems to be a proteid substance which may vary in its particular character from stage to stage in the process of fermentation, and in these granules the fat which may be demonstrated is deposited.

The structure of the cytoplasm varies. When the cells of *S. Ludwigii* of early stages of fermentation are hardened in Flemming's fluid and stained in Heidenhain's hæmatoxylin, we get an appearance like that illustrated in Figs. 36, 37, and 38. In these the cytoplasm is shown to contain a reticulum whose meshes are delicate and whose nodal points are thickened. In some cells, as for example in Fig. 38, the reticulum forms a ring around a cospuscle, whose nature will be discussed below. In other cases the reticulum is in intimate connection with the corpuscle. In corrosive sublimate preparations a reticulum is not readily observable, and this is due to the property the reagent has of fixing not only the reticular portion of the cytoplasm, but all the proteids in its meshes, whereas the acetic acid of Flemming's fluid dissolves out some of these. Indications, however, of a reticulum can be found if the stain of the iron-alum hæmatoxylin is carefully decolourized with weak iron-alum solutions.

When the cells have been prepared with iodine solution and stained with iron-alum hæmatoxylin the reticulum shown is coarser as a rule than in Flemming's fluid preparations, the meshes are larger and the trabeculæ thicker. I am inclined to regard this result as due to the iodine reagent which fixes the cytoplasm slowly, and consequently may permit plasmolytic alterations.

The presence of vacuoles affects only slightly the reticular structure, merely condensing the cytoplasm in their immediate neighborhood.

In corrosive sublimate preparations which have been carefully stained with Delafield's or Ehrlich's hæmatoxylin or with Meyer's hæmalum, the cytoplasm gives unmistakable evidence of the presence of chromatin diffused through it as well as localized at particular points. This diffuse distribution causes the whole cell to be deeply stained when one employs the ordinary staining reagents, in the concentrated form which is usual in the case of other cytological preparations. It is the most striking point that one finds when one for the first time makes preparations of yeast cells, and this being so it is surprising that little attention has been given to it in the literature of the subject.

In the cytoplasm as Errera¹ has shown, may be found glycogen, and it occurs in abundance in the cells of the later stages of fermentation. When iodine solution is applied to the cells from Pasteur solutions in the first nine hours after fermentation begins, very rarely only does it show the presence of glycogen, and then only in the form of minute granules. The slight brown tint which the cytoplasm in general at this time gives is due to the absorption of iodine by the cytoplasmic chromatin, and is not due to dissolved glycogen. In cells of from ten to thirteen hours of cultivation in Pasteur solutions the glycogen occurs in small masses in the cell. These masses, of which there is usually one to each cell, vary in size, and are more or less irregular in outline and placed adjacent to the cell membrane. In later stages the glycogenic mass may be so large as to occupy the greater part of the cell.

IV.—THE CHROMATIN-HOLDING STRUCTURES.

In yeast cells which have been hardened in Flemming's fluid or in corrosive sublimate solutions, and stained with very dilute solutions of Ehrlich's or Delafield's hæmatoxylin applied for from sixteen to twenty hours, one finds, as already pointed out, a slight colour, due to the presence of chromatin in the cytoplasm generally, and a very deep stain at one or more points in the cell. The latter may also be demonstrated by employing the iron-alum hæmatoxylin method for staining, but as it is not selective its action is less clearly indicative of the presence of chromatin, or of substances allied to chromatin, than that of the staining reagents mentioned. The diffuse stain which is given to the cytoplasm may serve to obscure the presence of a structure or structures which may be present.

One frequent type of this structure is, ordinarily, a spherical mass like that represented in Figs. 36, 37, and 38, and, as in these cases, varying somewhat in size. This body, which I may term, for the sake of brevity, the corpuscle, is, in the great majority of cells, homogeneous and dense, and it stains much more deeply than the cytoplasm generally. It is not, however, always present, for it appears to be absent in cells in the different stages of fermentation, and no method of hardening and staining the cells will demonstrate its presence in all. This has been admitted by Bouin and Buscalioni. The former observer tried, by deeply restaining cells which at first appeared to be free from corpuscles, to bring out the presence of the latter, but succeeded only in

¹ Report British Association, Bristol Meeting, 1898, p. 1,068.

a few of such cells, in which the corpuscles were found to be deficient in chromatin. Buscalioni believes that yeast cells exist which are free from these bodies. Sometimes, on the other hand, one finds yeast cells also which contain not only one but several corpuscles, each, however, smaller than the single corpuscle of other cells.

Very rarely in preparations of *S. cerevisiae* but very frequently in those of *S. Ludwigii* as cultivated in the sap of the ironwood tree (*Ostrya virginica*), the corpuscle instead of being spherical and homogeneous, is irregular in contour and consists of one or more deeply stainable, dense granules, imbedded in a substance which constitutes the greater part of the corpuscle, and which is less markedly affected by dyes. Sometimes the irregularities in the contour may be so great as to give the corpuscle a stellate appearance. Bouin observed corpuscles of similar shape and structure in *S. cerevisiae* and *S. pastorianus*.

The corpuscle is the nuclear body of Errera and Wager, and the nucleus of Moeller, Bouin, Buscalioni and others. As these authors describe it, it divides by a process which is a simple form of karyokinesis, and, therefore, it is in their view a fully developed chromatin-holding organ. According to Janssens and Leblanc the corpuscle is a nucleolus of a nucleus which can, by appropriate methods, be revealed as surrounding and containing the nucleolus. This nucleus further is provided with a membrane which becomes invisible when division of the nucleolus takes place, the caryoplasm also disappearing.

In preparations hardened with iodine solution and stained with dilute hæmatoxylin, a body like the nucleus of Janssens and Leblanc can be observed surrounding the "nucleolus," but it is found only in a small number of cells, whereas in the greater number the "nucleolus" or corpuscle lies free in the cytoplasm. In preparations also made with Flemming's fluid and stained with iron-alum hæmatoxylin, the corpuscle is rarely found to be included by a structure like that described by these authors, and when the latter is observed it contains no chromatin and does not give any evidence of structure in its interior. It is as such quite different from the corpuscle already referred to, to be found in *S. Ludwigii* when cultivated in sap. What it is I am not certain, but I am inclined to regard it as a vacuole which, placed above or below the corpuscle, may with the latter strongly resemble a nucleus and nucleolus. In iodine preparations stained with iron-alum hæmatoxylin, the structure in question may be absolutely unstained while the "nucleolus" or corpuscle, and the cytoplasm are deeply coloured. On the other hand, one may find a vacuole, whose wall is rich in stainable material, overlies

or underlie a corpuscle, giving exactly one of the conditions described and illustrated by Janssens and Leblanc.

The corpuscle does not in the fresh cell react with acetic-methyl green like a chromatin body. Preparations of this reagent, which would bring out clearly in fresh animal and vegetable cells the chromatin-holding structures, left the corpuscles of the yeast cell, even after hours, unaffected, while the cytoplasm stained readily. Another point of contrast is to be found in its affinity for eosin, which is more readily absorbed and retained by it than is hæmatoxylin.

In the throat yeast the corpuscle was, in the great majority of instances, more or less irregular in outline, always excentrically placed, and in the great majority of cells, in close contact with a vacuole. Some times it was crescentic in outline, the vacuole fitting into its concavity. In this form very little chromatin was found in the cytoplasm and as a consequence the corpuscle stood out quite clearly.

In *S. Ludwigii* as it grew in the sap of the iron-wood tree, fixation with Flemming's fluid and staining with iron-alum hæmatoxylin and eosin demonstrated the corpuscle frequently as a reddish body, having at times a tint of blue-violet and surrounded by a garland-like structure formed of deep blue-violet-stained chromatin. This structure, on closer analysis, is found to be formed of granules and elongated masses of chromatin. Sometimes it appears open or discontinued at one side, and, especially if the corpuscle is pear-shaped, its prolongation extends beyond the limits of the chromatin structure. Usually, however, in these preparations the structure is closely applied to the corpuscle. When there are two corpuscles in the cell there is a structure in question about each of them. Rarely the corpuscle appears separated from the cytoplasm, and, consequently, from the garland-like structure, by a zone of clear space. (Fig. 28).

I regard this garland-like structure formed of chromatin as quite the same as the membrane of fine granules closely applied to, and completely surrounding, the "nucleolus," as described by Wager, who found that the granules and nucleolus stained differently. In my preparations of *S. cerevisiæ* the membrane does not appear as uniform and as regular as Wager figures it; and its irregularity, and sometimes the absence of close contact between it and the corpuscle, remind one strongly of the conditions in *S. Ludwigii*. The point to note specially is the contrast in staining presented by the corpuscle and the structures surrounding it. The latter has a marked affinity for hæmatoxylin, while the former absorbs eosin readily. Sometimes, in corrosive sublimate preparations

stained with very dilute solutions of hæmatoxylin, the corpuscles may be unstained, or stained no more deeply than the cytoplasm generally. (Fig. 28).

In ordinary cultures of *S. Ludwigii* the garland-like structure may be so infrequently present in its typical form as to be overlooked. Then in many cells granules like those found by Wager and Bouin in *S. cerevisiæ* may be observed. This illustrates how much, as regards structure, is dependent on the mode of cultivation of the yeast cell.

When yeast cells, hardened in alcohol, were submitted at 37°C. to digestion in artificial gastric juice, made by dissolving some glycerine extract of pepsin in a 0.2 per cent. solution of hydrochloric acid, after forty-eight, seventy-two, and ninety-six hours there were very few evidences of the occurrence of corpuscles remaining, nor was there any stainable substance left in the cytoplasm. The corpuscles seem to be affected very much by the digestive solution, for even when the cells were deeply stained with the iron-alum hæmatoxylin, corpuscles were only rarely found, and then they appeared with a large vacuole in their interior (Figs. 39 and 41), or to have lost their stainable substance (Fig. 40). These traces of the corpuscle also disappeared after treatment of the preparations for 24 hours with a 0.1 per cent. solution of potassic hydrate. The results of the treatment with artificial gastric juice seem to indicate that the stainable substance in the cytoplasm and in the corpuscles is different from the chromatin of the nuclei of other organisms, which is unaffected by this fluid.

Similar results were obtained when fresh cultures of *S. cerevisiæ*, in Pasteur solution, were digested for forty-eight hours or more in artificial gastric juice, after which the cells were fixed with iodine solutions and hardened in alcohol. Only very rarely in these, even after the most careful application of the various methods of staining, and especially of the iron-alum hæmatoxylin process, did I succeed in finding traces of a corpuscle. The cells of such preparations seem to have lost their power to stain with Ehrlich's and Delafield's hæmatoxylin solutions, but to have increased their affinity for eosin.

The micro-chemical reactions are quite decisive as regards the relationship of the stainable substance. When the glycerine-ammonium sulphide method is employed to demonstrate the organic iron in cells of *S. Ludwigii* and *S. cerevisiæ*, the results obtained after ten days at the latest distinctly demonstrated the presence of "masked" iron in the one or more corpuscles which may be present in each cell in the walls

of the vacuoles, and in the cytoplasm generally (Figs. 42-49). The reaction is usually intense in the corpuscles, so much so that they may appear not dark green but black from the excess of ferrous sulphide developed in them by the reagent. The reaction in the cytoplasm is much less marked but distinct. It may be diffuse, but if the preparation, though successful, has been only a few days in the reagent, one may observe, in a majority of the cells, that the reaction is limited to the cytoplasmic network (Figs. 50 and 51). In some cells a series of granules constituting a membrane about the corpuscles like those already described, gives a very distinct reaction (Fig. 46). Sometimes the number and size of the corpuscles thus demonstrated suggest the character of granules, but there is, in the majority of such cases, one at least which is of the usual size (Figs. 45, 46, 47, and 49). The reaction in the wall of the vacuole may be very distinct, and especially in granules located in it. (Figs. 42, 43, 44, and 48).

On applying the method to demonstrate the presence of organic phosphorus, the later is found to be localized in the same manner as the organic iron. The corpuscle is rich in it, and the wall of the vacuole in contact with the corpuscle gives a distinct reaction for it, and at times specially in the granules found in it. Portions of the cytoplasm, which appear to correspond to the nodal points of the reticulum, give a deep reaction (Fig. 56).

It is evident from the presence of "masked" iron and organic phosphorus, both distributed in the yeast cell to an extent parallel with the distribution of the substance which stains with hæmatoxylin, that the organism contains in its corpuscle or corpuscles, in its cytoplasm, as sometimes in the wall of its vacuole, a substance closely related to the chromatin of higher organisms, but differing from the latter in the effect exercised on it by artificial gastric juice. The stainable substance found in the corpuscles further differs from ordinary chromatin in that it has no affinity for acetic-methyl green.

There remain now to be described structures which are, so far as my observations go, to be found only in *S. Ludwigii*, when cultivated in the sap of the iron-wood tree. The character of these structures is seen by an examination of Figs. 21-27, and 29, in which they are illustrated. They are not all of the same type. As in Figs. 21 and 29 one may observe a large nucleus-like body in which a network, somewhat like that found in a fully typical nucleus, exists. There may also be a corpuscle in this structure which simulates a nucleus. In some cases the structure in question may resemble a nucleus in the stage preparatory to the for-

mation of the chromosomes (Fig. 23). In other preparations, one may obtain cells in which a series of vacuoles is found in close contact with each other, and all surrounded by a substance which stains deeply in hæmatoxylin (Figs. 22 and 24). Rarely one sees structures like those illustrated in Figs. 26 and 27. In Fig. 25, there are in mother and daughter cells structures allied in general form to those found in Figs. 22 and 24.

A somewhat similar instance of vacuolation of chromatin-like masses may be observed in mycelium-like threads developing sometimes with the cells of *S. Ludwigii* in sap cultures. In these the vacuoles vary from an almost infinitesimal size to that of extraordinary dimensions (Fig. 58). The largest ones appear to be formed of a large number of vacuoles fused through the rupture of the more centrally placed partitions (Fig. 58*d*). In a few cases the fused vesicles may form a very large structure, presenting some resemblance to a nucleus (Fig. 57).

Whether these structures are formed of that variety of chromatin which is to be found in yeast cells cannot be decided as yet. The cells containing them are so few in any preparation made with the glycerine-ammonium sulphide method to show the distribution of organic iron, or with the nitric-molybdate reagent to determine the occurrence of organic phosphorus, that they must only very rarely be observed. In only one sulphide preparation did I see a cell which appeared to contain a structure like one of those in question (Fig. 52). In this case the iron reaction of the substance forming the structure was quite marked. It would seem to indicate that these structures are formed of yeast chromatin.

There can be no question about the nature of these structures. They certainly are not nuclei, either normal or degenerated. They owe their form and arrangement to a property of chromatin which, I believe, has not hitherto been regarded as characteristic of it. In the Cyanophyceæ, as already described, the chromatin-like substance not dissolved in the "central body" forms spherules, in the centre of each of which may be found a vacuole (Figs. 4, 6, and 11). In the Foraminifer, *Calcituba polymorpha* Roboz,¹ the nuclear chromatin before division, at first homogeneous, undergoes extensive vacuolation, and upon this process division depends. Indeed the structure of the nucleus ordinarily would appear to depend on the inherent power of chromatin to produce vesiculation or vacuolation, with the formation ultimately of a reticular

¹ F. Schaudinn, "Untersuchungen an Foraminiferen. I. *Calcituba polymorpha* Roboz," Zeit. für Wiss. Zool., Vol. LIX, p. 191, 1895.

arrangement. This vacuolation may sometimes be observed, in a marked degree, in the masses of chromatin from chromatolysed nuclei in higher animal and vegetable organisms, and it is manifested even in chromatin nucleoli in normal nuclei. This indicates that chromatin secretes fluid in certain conditions, and that vacuoles are formed by this secretion. In the cells of *Saccharomyces* under consideration, it is not certain, as was pointed out, that the structures which simulate nuclei are formed of a chromatin-like substance, but it is probable that they are constituted of it, and this would explain their appearance, although in plasmolysed chromatin a like richness of vacuolation has not yet been observed. I would regard these structures as caused by extensive vacuolation of chromatin-like masses formed in the cytoplasm.

V.—BUDDING AND SPORULATION.

In the process of budding a portion of the cytoplasm is forced into a diverticulum of the cell membrane, the quantity at first forced out being small, but eventually the bud may contain from one-third to one-half of the cell contents. It is only in this way that we can explain the "streaming out" appearance of the cytoplasm in the neck of the bud. We may see vacuoles elongated and extending into the bud (Figs. 30, 32, 42, 44 and 48), or one or more of Raum's granules having extended dumb-bell shapes, the extremities of which lie in the mother and daughter cells. Sometimes also one may find one of the peculiar reticulated, chromatin-like masses, described above, occupying, as a dumb-bell shaped figure, the neck of the bud. The pressure to which the cytoplasm of the mother cell is subjected and the narrow passage of the neck of the bud tends to draw out and elongate all the structures which are forced through the narrow neck.

These conditions are responsible for the elongation and constriction of the corpuscle as described by Bouin, Janssens and Leblanc, Wager and others, who regard these phenomena as constituting evidence of nuclear division. I have found in many instances that the corpuscle is thus divided between the mother and daughter cells. When the corpuscle is found in the neighbourhood of the commencing bud, it is, with the cytoplasm surrounding it, forced to the opening, which is rarely large enough to permit its passage, and if the diameter is large enough the cytoplasm that is driven with it prevents the corpuscle from passing through the opening. The corpuscle, being plastic like the cytoplasm, may completely fill the passage and project into the interior of the bud, its dumb-bell form being then quite marked. The constriction may

deepen until only a fine strand connects the two terminal spheres, and when the bud further develops there may be a complete separation of the two parts, one remaining in the mother cell, the other forming the corpuscle of the daughter cell. Sometimes, however, the whole corpuscle is forced through the neck into the bud. This is to be found not rarely in the sap cultures of *S. Ludwigii* and rarely in *S. cerevisiae*. Buscalioni believes that this happens sometimes in *S. guttulatus*, and Bouin found that it does occur in *Mycoderma cerevisiae*. The latter author would thus explain the absence of a nucleus from some cells. It is in this way, I believe, that the complete absence of a corpuscle in the mother cell and the presence of a large one in the daughter cell may be explained. I have also found the bud in a few cells of *S. Ludwigii* grown in sap to contain two small corpuscles, while the mother cell gave not the slightest evidence of the presence of a corpuscle. In these cases one of the daughter corpuscles, after their formation by constriction of the parent structure in the manner described, which should, as is usually the case, remain in the mother cell, is carried with the cytoplasm into the bud.

In *S. Ludwigii* buds may develop and separate without the constriction and division of the corpuscle of the mother cell. This is specially the case when the corpuscle is in a part of the cell remote from the commencing bud. Wager states that in this case the nuclear body (the corpuscle) makes its way to the opening of the mother cell into the bud and then begins to divide. This may happen, but I have found in a number of instances the bud full grown, while the corpuscle remained undivided in the remote part of the cell.

There can be but one interpretation of these facts. The elongation and constriction of the corpuscle are the results of purely physical forces and conditions, such as operate on the cytoplasm in the neighbourhood of the bud, and the constriction and resulting division of the corpuscle are not absolutely necessary factors in the development of the bud. The formation of two corpuscles out of one in this way can scarcely be regarded as a case of direct division or simple karyokinesis, as some observers have claimed it to be.

In *S. Ludwigii* many of the buds contain cytoplasm richer in "masked" or organic iron than that in the parent cell. This would indicate that the cytoplasm of the bud is richer in chromatin, and the results of staining with hæmatoxylin seem to support this view. It is not infrequently found that the cytoplasm lining that part of the membrane of the bud remote from the neck stains deeply and gives a deep reaction

for organic iron with the glycerine-sulphide method. Perhaps this distribution may be explained by supposing that the first cytoplasm to pass out into the developing bud is richer in chromatin-like substance.

Quite different is the action of the corpuscle in sporulation. Here, however, the cytoplasm also acts differently. When the cell is ready to sporulate the chromatin dissolved in the cytoplasm begins to concentrate in a zone about the corpuscle, the diameter of the zone diminishing as this stage advances, while the corpuscle appears to lose its distinctness. The concentration advances until all of the cytoplasmic chromatin is collected in a very narrow zone about the corpuscle which, in some cases, may appear very finely granular. At this stage occurs an elongation of the corpuscle and its enclosing body of cytoplasmic chromatin, the elongation rarely being to the full length of the cell. The central portion constricts or becomes more and more slender, until separation of the more or less rounded extremities occurs. Thus two corpuscles are formed, each with a very narrow enclosing zone of cytoplasmic chromatin. Each of these now elongates and divides as the parent structure does. In the second division, however, the cytoplasmic chromatin seems to disappear, or perhaps is taken up into the corpuscle.

My observations on the whole agree with those of Wager, but I have never been able to find the granules which form in the corpuscle or nucleolus immediately before and during its elongation, as described and illustrated by that observer. Nor can I corroborate his view that the nuclear vacuole, which exists in the cell previous to sporulation, divides and redivides many times, thus distributing the chromatin through the cytoplasm, which has, in consequence, a delicate foam-like structure. So far as my observations go, the chromatin in the cytoplasm before the stage of sporulation commences, is not different in its character or distribution from that found in the ordinary yeast cell, for example, during budding.

According to Janssens and Leblanc, the act of sporulation is preceded by a division of the nucleus and its nucleolus, followed by a fusion of the two nuclei thus produced. These authors believe that this fusion or conjugation constitutes sexual fertilization. They find that the two nuclei formed disappear, and in their place, one only, whose nucleolus is large and distinct, is observed. It is rather difficult to accept this interpretation. What they claim to have observed as constituting a nucleus may, as I have pointed out, be found in some cells only, and their nucleolus is the corpuscle, two or more examples of which may some-

times be found in the cell. It is, therefore, not impossible to find frequently in cells in cultures beginning the sporulation stage two small corpuscles. It is, however, another matter to prove that these corpuscles fuse to constitute a single large corpuscle, such as may be found in other cells of the same preparation in which no evidence of fusion having occurred can be observed.

According to these authors, a spindle formed of very fine parallel threads constitutes the connecting strand in the division of what they term the nuclei in sporulation. I have never been able to observe such a structure, but it is possible that what is found in the varieties of yeast used for observing it by Janssens and Leblanc, may permit the demonstration of such a spindle more readily than in the forms I employed. I must say, however, that in *S. Ludwigii*, employed by them also for this object, I was unable to find anything resemble a spindle of fine threads.

I found in a number of cells of *S. cerevisia*, a structure which resembles very much that described by Janssens and Leblanc, and compared by them to a cell plate. It was a line formed of delicate, closely placed granules running transversely to the strand connecting the two developing corpuscles and completely dividing the cell into two halves. The dotted line was in the majority of cases so fine and so difficult to see properly that it required the best illumination and the highest available magnification to bring it out. Whether it is to be regarded as a cell plate cannot at present be determined.

The division found in sporulation can scarcely be described as a simple form of karyokinesis. It is rather to be compared to the division of a chromatic filament in the formation of two chromosomes. A more analogous case is that of the division of the nucleolus in *Euglena viridis*, as described by Blochman¹ and Keuten². In this form the nucleolus, at the commencement of division, does not disappear as it ordinarily does in other cells, but remains in all the stages. When the chromosomes which are formed in the normal way begin to constitute the dyaster stage, the nucleolus elongates into a dumb-bell figure, the constriction first observed deepening, until complete separation of the spherical ends takes place. Each of these passes into the corresponding daughter nucleus. In this case, while the ordinary chromatin of the nucleus undergoes division by the karyokinetic method, the nucleolus undergoes direct division. In sporulation in *Saccharomyces* it is difficult

¹ "Ueber die Kernteilung bei *Euglena*." Biol. Centralbl., Vol. XIV, p., 194, 1894.

² "Die Kernteilung von *Euglena viridis* Ehrenberg." Zeit. für Wiss. Zool., Vol. LX, p. 215, 1895.

to believe that anything more complex occurs than this nucleolar division.

If, on the other hand, the division in sporulation is regarded as karyokinetic, it must be also held to be an exceedingly rudimentary type of that process. It would in fact have to be admitted as differing so little from what is called direct division as to be almost indistinguishable from it.

SUMMARY.

1. In *Saccharomyces* the cytoplasm is usually finely reticulated, and contains one or more vacuoles. It takes a diffuse stain with hæmatoxylin, and gives a diffuse reaction for "masked" iron and organic phosphorus.

2. In addition to the chromatin-like substance diffused throughout the cytoplasm, there is usually a more or less homogeneous, spherical body in the cell, the corpuscle, the "nucleus," "nucleolus," and "nuclein body" of various observers, which stains specially with hæmatoxylin, and gives the reactions for "masked" iron and organic phosphorus, but does not stain with acetic-methyl green. This body is neither a nucleus nor a nucleolus. Several examples of it, though of small size, may be present in a cell. On the other hand, cells are found without a trace of a corpuscle.

3. The chromatin-like substance differs from the chromatin of higher animal and vegetable cells in being soluble in artificial gastric juice.

4. When budding begins, the corpuscle, if placed adjacent to the point where the bud is developing, becomes elongated and constricted in its middle portion. One end of the elongated structure may be forced into the neck of the bud, and when the constriction is completed by separation of the two halves, the daughter cell may thus receive a corpuscle. Both daughter corpuscles may pass into the bud, leaving the mother cell without a corpuscle. A complete bud may be formed without such a division of the corpuscle taking place, and thus the daughter cell may commence independent life without a corpuscle.

5. In sporulation the cytoplasmic chromatin collects in the immediate neighborhood of the corpuscle, which also undergoes certain granular changes, then elongates with constriction in its central part. Each of the two daughter corpuscles thus formed repeats this process of division one or more times, the daughter corpuscles resulting ultimately forming the corpuscles of the spores.

6. The division of the corpuscles in budding is a purely mechanical result, and is not essential to the formation of the bud. The division preparatory to sporulation is apparently a functional act. It is not of the nature of true karyokinesis, and it may be compared to the division of the nucleolus in *Euglena viridis*.

7. In cells of *Saccharomyces Ludwigii*, from a culture in sap, one finds, rarely, structures which strikingly remind one at times of a nuclear organ. These structures are apparently formed of a chromatin-like substance, and their existence is due to a property which chromatin possesses of forming vacuoles in itself. When the vacuoles are fully formed, the portions of chromatin-like substance separating the vacuoles may be so delicate as to suggest the occurrence of a network like that of a nucleus.

Since the foregoing paper was written, the results of an investigation by Ascoli,¹ conducted in Kossel's laboratory on plasmic acid, a variety of nucleic acid, have been published. The preparation of plasmic acid examined was obtained from yeast cells, and was found to contain about 1 per cent. of iron in a "masked" or organic form. This confirms what I have hitherto advanced, and what I have described in this paper, regarding the presence of "masked" iron in combination with a nucleic or chromatin compound in yeast cells.

¹ "Ueber die Plaminsäure," Zeit. für Physiol. Chemie, Vol. XXVIII, p. 426, 1899.

EXPLANATION OF PLATE.

All the Figures were outlined from the objects with an Abbe camera lucida and as viewed with a 3mm., 2mm., or 1.5mm. apochromatic oil immersion objective, and an 8, 12 or 18 compensation ocular (Zeiss).

- FIG. 1.—*Cylindrospermum majus*. Fresh, 48 hour's culture in the laboratory. $\times 2,000$.
- FIG. 2.—*Oscillaria Froehlichii*. A cell viewed from the flat surface. Picric acid, picrocarmine, hæmatoxylin. $\times 3,000$. The transition from the central body to the peripheral zone, as represented in the figure is, unfortunately, rendered too abrupt.
- FIG. 3.—*Oscillaria Froehlichii*. View of two cells from the side, optical section. Picric acid, picrocarmine, hæmatoxylin. $\times 3,000$.
- FIG. 4.—*Tolypothrix* sp. Corrosive sublimate, hæmatoxylin. $\times 1,500$.
- FIG. 5.—*Tolypothrix* sp. Digestion with artificial gastric juice three days, extracted with alcohol and ether, Ehrlich's hæmatoxylin (dilute) 24 hours. $\times 1,500$.
- FIG. 6.—*Tolypothrix* sp. Strong Flemming's fluid 24 hours, Delafield's hæmatoxylin. $\times 1,500$.
- FIG. 7.—*Tolypothrix* sp. Corrosive sublimate, Delafield's hæmatoxylin. $\times 1,500$.
- FIG. 8.—*Oscillaria natans*. Digested with artificial gastric juice 6 days, alcohol 1 day, alcohol and ether 8 hours, hæmatoxylin. $\times 1,500$.
- FIG. 9.—*Oscillaria Froehlichii*. Digested with artificial gastric juice 6 weeks, alcohol and ether, KHO 0.3 per cent. 24 hours, picrocarmine. $\times 1,360$.
- FIG. 10.—*Microcoleus terrestris*. Digested with artificial gastric juice 3 days, hæmatoxylin, glycerine. $\times 1,500$. The dye has given a greenish-blue tinge to the cytoplasm in the central part.
- FIG. 11 a and b.—*Microcoleus terrestris*. Alcohol, a, hydrogen proxide 3 hours, acid ferrocyanide solution, balsam; b, hæmatoxylin, glycerine. $\times 1,500$.
- FIGS. 12 and 13.—*Tolypothrix* sp. Alcohol, hydrogen peroxide 3 hours, acid ferrocyanide solution, glycerine. $\times 3,000$.
- FIG. 14.—*Oscillaria Froehlichii* (?). Alcohol, hydrogen peroxide 3 hours, acid ferrocyanide solution, picrocarmine, balsam. $\times 3,000$.
- FIG. 15.—*Lyngbia* sp. Picric acid, picrocarmine. $\times 1,000$.
- FIG. 16.—*Oscillaria tenerima*. Picric acid, picrocarmine, hæmatoxylin. $\times 3,000$.
- FIG. 17.—*Tolypothrix* sp. Picric acid, hæmatoxylin, balsam. $\times 1,334$.
- FIG. 18 a and b.—*Cylindrospermum majus*. Old culture, acetic-methyl green. $\times 3,000$.
- FIG. 19.—*Oscillaria natans*. Fresh culture, artificial gastric juice 48 hours, picrocarmine, glycerine. $\times 2,250$.
- FIG. 20.—*Tolypothrix* sp. Alcohol, nitric-molybdate 10 hours. Phenylhydrazin hydrochloride, balsam. $\times 1,334$.

- FIGS. 21-29.—*Saccharomyces Ludwigii*. Corrosive sublimate 8 hours, alcohol, Delafield's hæmatoxylin (very dilute) 17 hours. $\times 3,000$.
- FIGS. 30-33.—*Saccharomyces Ludwigii*. Alcohol, Delafield's hæmatoxylin (very dilute) 48 hours, balsam. $\times 2,400$.
- FIGS. 34-35.—*Saccharomyces Ludwigii*. Corrosive sublimate, alcohol, Delafield's hæmatoxylin (very dilute) 18 hours. $\times 2,400$.
- FIGS. 36-38.—*Saccharomyces Ludwigii*. Flemming's fluid, iron-alum hæmatoxylin, balsam. $\times 2,000$.
- FIGS. 39-41.—*Saccharomyces Ludwigii*. Artificial gastric juice, 96 hours, iron-alum hæmatoxylin, eosin, balsam. $\times 2,000$.
- FIGS. 42-49.—*Saccharomyces Ludwigii*. Alcohol, glycerine and ammonium hydrogen sulphide 10 days. $\times 3,000$.
- FIGS. 50-51.—*Saccharomyces Ludwigii*. Alcohol, glycerine and ammonium hydrogen sulphide 6 days. $\times 2,250$.
- FIGS. 52-55.—*Saccharomyces Ludwigii*. Alcohol, glycerine and ammonium hydrogen sulphide 10 days. $\times 2,250$.
- FIG. 56.—*Saccharomyces Ludwigii*. Alcohol, nitric-molybdate 5 hours, phenylhydrazin hydrochloride, balsam. $\times 3,000$.
- FIGS. 57-58 *a-d*.—Mycelial forms growing with *Saccharomyces Ludwigii* in sap cultures. Corrosive sublimate, hæmatoxylin, balsam. $\times 3,000$.
- FIG. 59 *a-c*.—*Beggiatoa alba*. "Cocci," "Comma," and "Spirillum" forms. Corrosive sublimate, hæmatoxylin. $\times 3,000$.
- FIG. 60.—*Beggiatoa alba*, "Leptothrix" forms. Picric acid, picrocarmine, hæmatoxylin. $\times 3,000$.
- FIG. 61.—*Beggiatoa mirabilis*. Alcohol, nitric-molybdate, 5 hours, phenylhydrazin hydrochloride, balsam. $\times 1,334$.

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